Transfer Technique - Seeing where you are going

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Introduction

The main variables that affect nidation are related to uterine receptivity, embryo quality and the efficiency of embryo transfer. Embryo transfer is the critical step in assisted reproductive technology; with rigid catheters, contamination with blood, mucus or bacteria, increased contraction waves of the myometrium, and the level of difficulty in introducing the catheter inside the uterine cavity tending to reduce embryo implantation rates (1-9). Little attention has been paid to embryo transfer, a fact reflected in the scientific publications regarding this subject, which are rare compared with those evaluating other aspects of IVF. The probable reason is the apparent simplicity of this maneuver, since most clinicians do not consider inserting a catheter through the uterine cervix and ejecting embryos a difficult task. This facility, however, is relative. Differences in individual transfer performances are reflected in the results reported in the literature. Meldrum et al. (10) and Naaktgeboren et al. (11) emphasized that meticulous embryo transfer is essential for the success of IVF. Assessing the question of the operator within the same program, Hearns-Stokes et al. (12) found significant differences in pregnancy rates between 11 clinicians who performed 854 embryo transfers. In that study, all aspects of the IVF-embryo transfer cycle were standardized: the groups were homogenous in terms of all aspects including embryo quality, the number of embryos transferred and the transfer technique employed. Karande et al. (13) reported significant differences in pregnancy rates between clinicians even when uniform protocols of ovarian stimulation and embryo culture were used. Embryos are routinely transferred through the transcervical route, with the catheter being inserted in two ways: blindly by 'clinical touch' or guided by ultrasound. Many services use the 'sensitivity' of the clinician to place the embryos within the uterine cavity at a point close to the fundus (4, 14),

similar to the description published by Edwards more than 20 years ago. With respect to this type of embryo transfer, which is more traditional, no attempt has been made to document the variables that might have a negative impact and cause low pregnancy rates and failure of the whole process, such as inadvertent touch of the catheter tip on the fundal endometrial surface or inappropriate embryo placement in the uterine cavity (6-8,15).

Seeing is Believing

Ultrasonographic observation has many potential advantages: it prevents touching the fundus of the uterus, it confirms that the catheter is beyond the internal os and it permits guidance of the catheter along the endometrial line, a fact that facilitates the use of more flexible catheters. In addition, the full bladder required for transabdominal ultrasound itself is useful for the correction of uterine access through the cervical route in cases of pronounced anteversion– anteflexion. The use of ultrasound to assist embryo transfer was first described by Strickler et al. (16), who reported that guided transfer is easier and less associated with catheter distortion. Later, several other studies showed that ultrasound-guided embryo transfer yields better implantation and pregnancy rates (6-9,17-22) in addition to facilitating the transfer procedure. Furthermore, ultrasound provides new insights into the process of embryo transfer. One interesting aspect is the site in the endometrial cavity where the embryos are placed, with some reports correlating this variable with the site of implantation. Baba et al. (23) analyzed 60 embryo transfers that resulted in 22 pregnancies and 32 gestational sacs. Twenty-six of the 32 sacs were detected by three-dimensional ultrasound in the area where the air bubble had been observed immediately after transfer. Liedholm et al. (24) placed small spheres in a column containing 50 micro liters of fluid and performed a simulated embryo transfer immediately before hysterectomy. The uterine cavity was then inspected and

the microspheres were found within a distance of 1 cm from the presumed deposition site. These results emphasize the importance of the site where the embryos were transferred. It has been traditionally accepted that embryos should be placed 5-10 mm below the surface of the uterine fundus. However, some investigators have suggested that placing embryos lower in the endometrial cavity may improve pregnancy rates. Waterstone et al. (25) reported the results of embryo transfer performed by two clinicians who followed different techniques. The first introduced the catheter until he felt the fundus and then pulled it back 5 mm before injecting the embryos, and achieved a final pregnancy rate of 24%. The second clinician introduced the catheter until a depth of 5 cm from the external os of the cervix and deposited the embryos without touching the fundus, and obtained a pregnancy rate of 46%. When the first clinician modified his technique according to that of the second, improvement in pregnancy rates was observed. Coroleu et al. (20) analyzed 180 patients submitted to guided embryo transfer, with the transfers being divided into three groups according to the distance between the uterine fundus and the site of embryo placement: group 1, 10 +/- 1.5 mm; group 2, 15 +/-1.5 mm; and group 3, 20 +/- 1.5 mm. The best implantation and pregnancy rates were observed for groups 2 and 3, in which the distance from the uterine fundus was greater than in group 1. Frankfurter et al. (26) retrospectively analyzed 23 patients who underwent two cycles of ultrasound-guided embryo transfer each, considering for each patient a transfer that resulted in pregnancy and one that did not. The results showed better pregnancy rates when the site of embryo placement relative to the length of the endometrial cavity was more distant from the uterine fundus. Among the various aspects of embryo transfer, the site of embryo placement in the uterine cavity has been postulated to influence embryo implantation rates. Whereas some investigators have suggested that improved embryo transfer results are obtained when the embryos are placed at lower levels in the uterine cavity (2, 11, 15, 20, 25, 26); others believe that higher levels in the endometrial cavity closer to the uterine fundus lead to higher rates (10, 27). Finally, some authors (28-31) postulate that the question regarding the site of embryo transfer is of no importance since it does not influence implantation as long as embryos are placed in the upper half of the cavity. However, many of these studies were based on retrospective observations and/or were not supported by ultrasound at the time of transfer.

Karande et al. compared the performance of a new coaxial catheter system with an echo-dense tip (Cook Echo-Tip catheter) with a Wallace catheter during ultrasound-guided ET. The echo-dense tip of the Cook Echo-Tip catheter was consistently seen with ultrasound guidance, minimizing the need for catheter movement to identify the tip. Implantation rate (30% vs. 35%), clinical pregnancy rate (57% vs. 55%), and ongoing pregnancy rate (49% vs. 47%) were similar in both groups (13).

Seventy-five ETs were performed using a 3D US scanner to locate the catheter tip in the uterine cavity. Three-dimensional ultrasound could show the exact position of the tip of the catheter in the uterine cavity quickly enough in most cases. It should be used in ET for seeking an optimal transfer area in the uterine cavity to assist in achieving high success rates and less complications (23).

Sallam et al. set up a study to determine whether moulding the embryo transfer catheter according to the uterocervical angle measured by ultrasound could improve pregnancy and implantation rates. 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. 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 degrees) had significantly lower pregnancy rates compared with those with no angle [OR = 0.36, 95% CI (0.16-0.52)]. The study observed that moulding the embryo transfer catheter according to the uterocervical angle measured by ultrasound increased clinical pregnancy and implantation rates and diminished the incidence of difficult and bloody transfers (32).

A prospective randomized (computer-generated random table) trial was performed to compare embryo transfer under abdominal US guidance (n = 255 women) with clinical touch embryo transfer (n = 260) (33). The clinical pregnancy rate was 26.3% (67/255) in the US-guided transfer group compared with 18.1% (47/260) in the clinical touch transfer group (P < 0.05). The implantation rate was 11.1% (100/903) in the US group compared with 7.5% (66/884) in the clinical touch group (P < 0.05). US-guided transfer was associated with a decrease in the difficulty of the transfers: 97% of transfers were easy in the US-guided group compared with 81% in the clinical touch group (P < 0.05). US-guided embryo transfer increased pregnancy and implantation rates in IVF cycles, as well as the frequency of easy transfers. It is suggested that the decrease in cervical and uterine trauma can play a role in the increase in prgnancy rates associated with US-

TABLE 1 - THE RELATIVE IMPORTANCE OF FACTORS IMPORTANT FOR SUCCESSFUL EMBRYO TRANSFER*.

Priority	Mean score#
Removal of hydrosalpinges	6.8
Absence of blood or mucus	6.6
Type of catheter	6.1
Not touching fundus	5.8
Avoiding tenaculum	5.7
Removal of all mucus	5.2
Ultrasonography of cavity before procedure	4.3
Leaving catheter in place for 1 minute	4.2
30 minutes of bed rest	3.8
Trial transfer	3.1
Ultrasonographic monitoring	2.6
Antiprostaglandins to prevent uterine contractions	1.9

*Data from reference 4
The possibile score for eache factor was on a scale of 1 to 10

guided transfer (33).

Anderson et al. published a paper to determine the effect of transvaginal ultrasound-guided ET in IVF cycles performed on patients who had previously failed to conceive from IVF and compare the results to previous cycles where ultrasound guidance was not used (34). There was no difference in any of the clinical parameters measured in IVF cycles resulting in pregnancy when transvaginal ultrasound-guided ET was used compared to the failed cycles when there was no ultrasound guidance. Of the patients who previously had failed IVF cycles and subsequently had IVF cycles with ultrasound guidance, those who became pregnant had higher mean embryo scores than those who did not become pregnant. Overall implantation and pregnancy rates were higher during the study period when transvaginal ultrasound guidance was used than in the previous 3 years when it was not used. The authors concluded that transvaginal ultrasound-guided ET may be responsible for successful IVF cycles in patients who had previously failed to conceive when embryos were transferred by the clinical touch method. Transvaginal ultrasound guidance may also be responsible for an overall increase in embryo implantation and pregnancy compared to the use of the clinical touch method (34).

A recent prospective randomized study investigated the influence of the depth of embryo replacement on the implantation rate after embryo transfer carried out under transabdominal ultrasound guidance (20). A total of 180 consecutive patients undergoing ultrasound-guided embryo transfers were randomized to three study groups according to the distance between the tip of the catheter and the uterine fundus at the moment of the embryo deposition in the lumen of the endometrial cavity: group 1: 10 +/- 1.5 mm; group 2: 15 +/- 1.5 mm; group 3: 20 +/- 1.5 mm. The position of the catheter tip in relation to the fundal endometrial surface in groups 1 (10.2 +/- 0.9 mm), 2 (14.6 +/- 0.7 mm) and 3 (19.3 +/- 0.8 mm) was significantly different. Implantation rate was significantly higher (P < 0.05) in groups 2 (31.3%) and 3 (33.3%) compared with group 1 (20.6%). The depth of the embryo replacement into the uterine cavity may influence implantation rates, and thus it should be considered as an additional procedure among factors recently proposed as associated with successful embryo transfer after IVF (20).

Variables influencing the Success of Embryo Transfer

The factors involved in achieving an effective transcervical intrauterine embryo transfer are the technical ability and training of personnel, catheter choice, value of a previous 'dummy transfer' and the need to minimize trauma during transfer and so prevent damage to the uterine lining, bleeding and uterine contractions. These factors can each negatively impact on pregnancy rates. The technique of embryo transfer is very crucial and great attention and time should be given to this step. In order to optimize the embryo transfer technique, several precautions should be taken. The first and most important is to avoid the initiation of uterine contractility. This can be achieved by the use of soft catheters, gentle manipulation and by avoiding touching the fundus. Secondly, proper evaluation of the uterine cavity and utero-cervical angulation is very important, and can be achieved by performing dummy embryo transfer and by ultrasound evaluation of the utero-cervical angulation and uterine cavity length. Another important step is the removal of cervical mucus so that it does not stick to the catheter and inadvertently remove the embryo during catheter withdrawal (5).

It is essential to deposit embryos as gently as possible during IVF, avoiding maneuvers that might trigger uterine contractions, which could adversely affect the results of this treatment. The time during which the embryo transfer catheter remains in the cervical canal might be related to stimulation of contractions. A recent study investigated the influence that the time interval before withdrawal of the catheter after ultrasound (US)-guided embryo deposit might have on the pregnancy rate in patients under IVF cycles. A total of 100 women about to undergo transfer of at least two optimal embryos was studied. The women were prospectively randomized into two groups: (i) slow withdrawal of the catheter immediately after embryo deposit (n = 51); and (ii) a 30 s delay before catheter withdrawal (n = 49). The pregnancy rates for

TABLE 2 - OUTCOME OF EMBRYO TRANSFER WITH AND WITHOUT ULTRASONOGRAPHIC GUIDANCE.

	Without ultrasonography			With ultrasonography			
Study (ref)	N. of patients	Implantation rate (%)	Clinical pregnancy rate (%)	No. of patients	Implantation rate (%)	Clinical pregnancy rate (%)	P value*
Wood et al. 22	260	14.1	25.4	258	19.9	38.4	<.022, .001
Coroleu et al. 57	180	18.1	33.7	182	25.3	50	<.01, .002
Prapas et al. 65	71	--	22.6	61	--	36.1	<.05
Lindheim et al. 19	35	17.5	35	67	27	61	<.05, .05

*Significance of implantation rate and pregnancy rate, respectively.
#NS = not significant

transfer in the two groups were 60.8 and 69.4% respectively, with no significant differences. There were no statistically significant differences in pregnancy rates between the two patient groups. The results indicate either that the waiting interval was insufficient to detect differences, or that the retention time before withdrawing the catheter is not a factor that influences pregnancy rate (35).

In order to determine the importance of ET in ART, the degree of difficulty of 4807 embryo transfers after IVF/ICSI was analyzed retrospectively (36). Logistic regression analysis identified the age of the subject, type of treatment (IVF versus ICSI), number of embryos transferred and degree of difficulty of embryo transfer as independent factors predicting pregnancy. The main focus of the study was to evaluate the importance of the difficulty of embryo transfer after taking into account the other confounding variables. Embryo transfer was classified as easy (2821), intermediate (1644) or difficult (342). The transfer was considered difficult if it was time consuming, the catheter met great resistance, there was a need to change the catheter, if sounding or cervical dilatation was needed or if blood was found in any part of the catheter. Easy or intermediate transfers resulted in a 1.7-fold higher pregnancy rate than difficult transfers (P < 0.0001; 95% confidence interval: 1.3-2.2). This study demonstrated that the degree of difficulty of embryo transfer is an independent factor as regards achieving pregnancy after IVF/ICSI. All efforts should be made to avoid difficult embryo transfers. Physicians should be alert to the factors associated with embryo transfer and should be instructed to use a stepwise approach in difficult transfers (36).

Applying a tenaculum to the cervix is a common practice when the correction of uterine position prior to embryo transfer is required. Lesny et al. conducted a study to assess junctional zone contractility before and after this procedure in 20 patients at the time of mock embryo transfer (mid-luteal phase, at commencement of down-regulation). Real-time transvaginal ultrasound and computer technology was used to evaluate the contraction pattern and frequency. When a tenaculum was applied, the total number of contractions, the number of cervico-fundal, random and opposing contractions all increased significantly (P values 0.0003, 0.005, 0.001 and 0.007 respectively). Eleven women displayed cervico-fundal contractions, prominent opposing and random contractions were observed in all 20 patients and four patients generated fundo-cervical waves not seen in any case before stimulation with the instrument. The authors concluded that manipulation with a tenaculum in the cervical area stimulates junctional zone contractions and is best avoided at the time of embryo transfer (3).

Marconi et al. set up a study to visualize by microhysteroscopy any possible lesions on the endocervix and endometrium made by the catheters commonly used for embryo transfer (ET). Twenty-three infertile patients underwent a mock transfer before a microhysteroscopy during the postovulatory phase (days 2-5 after ovulation) of the cycle with a Tomcat catheter (n = 5), Frydman's catheter (n = 5), Frydman's set (n = 3), or Wallace's catheter (n = 10). The lesions in all 23 patients were described and documented (tunnel-like, groove-like, punch-out, crater-like). The Wallace catheter appears to be less traumatic to the endometrium (but it seems that it is important to take care to not pass the internal os with the outer sheath). The Tomcat catheter and the Frydman's set caused the more significant lesions that

TABLE 3 - PROTOCOL FOR EMBRYO TRANSFER, BASED ON KEY FACTORS ASSOCIATED WITH SUCCESS.

Pre-ET trial transfer.

Transabdominal ultrasonographic guidance with full bladder.

Cervical lavage with culture media to remove excess mucus.

Practice transfer just through internal os.

Wallace catheter, 40 microliter volume with 10 microliter airbubble.

Gentle insertion: manipulate cervix with Pedersen or graves speculum.

Use ultrasonography to avoid catheter tip disrupting endometrium: avoid touching fundus.

Inject embryos slowly 1.5 cm - 2.0 cms from fundus as confirmed by ultrasonography.

Withdraw catheter slowly and turn through 90 degrees while withdrawl with piston depressed.

Inspection of catheter by embryologist for blood, mucus, or retained embryos.

were observed. In this preliminary study, for the first time endometrial lesions caused by the ET catheters were directly visualized and documented (37). Some of these observed lesions appear to be capable of compromising the success of ET (37). Murray et al. reported the effects of embryo transfers on endometrial integrity as assessed by direct hysteroscopic visualization. Subjects (n = 30) were patients of reproductive age undergoing diagnostic hysteroscopy. A mock embryo transfer was performed by a single clinician, immediately followed by saline hysteroscopy using a 2.7 mm hysteroscope. Hegar dilators or uterine sounds were not used. Representative video clips were recorded for independent assessment of endometrial integrity. Outcomes measured were ease of transfer (easy, moderate, difficult, very difficult) and details of the transfer technique. Endometrial damage was independently assessed and graded as follows: none, minor, moderate or severe. Of the easy transfers, 54% showed no endometrial damage. However, 37% showed moderate to severe damage in the easy transfer group. Of the moderately difficult transfers, there was no clear association between perceived difficulty of transfer and amount of endometrial damage. Clinical perception of ease of transfer does not correlate well with the degree of endometrial disruption (P = 0.41) (38).

Air Bubble Movement during ETs

In 1961, Iffy published a paper arguing the point that the usual mechanism of tubal ectopic pregnancy is expulsion of the embryo from the uterine cavity into the salpinx at the time it is ready to implant (39). This was largely ignored until the era of in vitro fertilization (IVF) and embryo transfer when Steptoe and Edwards (40) and Tucker et al. (41) each reported that their first embryo transfers after IVF had resulted in ectopic pregnancies. Initially, the high transfer volume used was thought to promote embryos being flushed into the Fallopian tubes at the time of embryo transfer. The volume was gradually reduced especially as Knutzen et al. (42) demonstrated that as little as 50 micro liters radio opaque dye injected into the uterine cavity could later be found in the Fallopian tubes in 44% of their patients. As indications for IVF broadened, it became more noticeable that tubal factor infertility was a risk factor (43). This further supported the 'reflux' theory: Embryos placed in the uterine cavity and subsequently reaching the Fallopian tubes would implant there if they, by some pathological process, were prevented from returning to the uterus. However, the question is whether the position of the catheter tip, the transfer volume used, or number of embryos replaced, would promote embryos being washed into the tubes at the time of transfer or whether an altered hormonal environment changes the uterine and tubal contractility and thus favors 'migration' of correctly placed embryos into the tubes (44). The discussion of how the embryos reached their ectopic sites has been hampered by the fact that no data are available in the literature. Embryo transfer is a 'blind' procedure in most major centers, where the operator guesses by the feel of the catheter tip where to place the embryos. Woolcott and Stanger (15) addressed the blind approach to embryo transfer and found that in 17.4% of cases their embryo transfer catheter unintentionally abutted the fundal endometrium and that in 7.4% of cases the guiding catheter was adjacent to the internal orifice of the Fallopian tube. They did not comment on ectopic pregnancy rates. Following the report of Yovich et al. (45) of increased numbers of ectopic pregnancies with placement of embryos high in the uterine cavity, it has been our routine to place the tip of the transfer catheter in the low mid-cavity.

Sieck et al. analyzed 3543 low mid cavity embryo transfers where a maximum of four embryos were replaced on the second or third post-ovum retrieval day (46). The embryos were suspended in a total of 25 micro liters of transfer medium in a Wallace ET catheter. Under the guidance of abdominal ultrasound, the tip of the catheter was introduced into the lower mid-cavity of the uterus and the transfer column slowly released here. The luteal phase was supported by progesterone. Clinical pregnancies were confirmed by demonstrating an intrauterine gestational sac or adnexal mass by ultrasound. The procedure resulted in 328 clinical pregnancies of which 11 (3.3%) were ectopic pregnancies. The overall indications for IVF were tubal factor infertility in 49% of

patients. All but one of the ectopic pregnancies (91%) had tubal pathology as indication for their inclusion in the IVF programme; the remaining pregnancy was a cervical pregnancy. The rate of ectopic pregnancies in the tubal group was 6.3% per embryo transfer. In all cases, the embryos had been placed correctly. The two air bubbles in the transfer column were often seen to make their way slowly up to the fundus of the uterus after a short phase of 'resting'. This was also seen to occur when the uterus was retroverted with the fundus below the level of the internal os indicating some active form of transport mechanism. Only on rare occasions did the authors see air bubbles remaining at the deposition spot or moving towards the cervix. Various authors have incriminated different stimulation protocols. Abnormal uterine contractility and altered tubal motility and cilia movements could be the result of very high estrogen concentrations and/or the anti-estrogenic effect of clomiphene citrate.

Transvaginal ultrasound-guided embryo transfer was performed on 121 consecutive patients. Observation was made of guiding cannula and transfer catheter placement in relation to the endometrial surface and uterine fundus during embryo transfer. The position and movement of a transfer-associated air bubble and the impact of sub endometrial myometrial contraction leading to endometrial movement was observed. Results indicate that tactile assessment of embryo transfer catheter placement is unreliable: in 17.4% of transfers the outer guiding catheter inadvertently abutted the fundal endometrium. The outer guiding cannula indented the endometrium in 24.8% and the transfer catheter embedded in the endometrium in 33.1%. Unavoidable sub-endometrial transfers occurred in 22.3% of transfers. Ultrasound-guided transfer avoided accidental tubal transfer in 7.4% of transfers. Transfer catheter withdrawal did not significantly affect embryo transfer-associated air bubble position. Endometrial movement due to sub-endometrial myometrial contraction was obvious in 36.4% of cases, with active motion of the transfer-associated air bubble occurring in 28.1%. Pregnancies occurred in 45.5% of transfers with endometrial movement compared to 15.6% ($P < 0.001$) without (15).

Woolcott et al. investigated whether standing upright shortly after embryo transfer has any potential to affect the position of embryos transferred to the uterine cavity during treatment with in-vitro fertilization (IVF) (47). This was assessed by ultrasound-guided tracking of embryo-associated air within the uterine cavity. A prospective study of 93 patients undergoing 101 consecutive embryo transfers in an IVF programme was carried out. Transvaginal ultrasound guided embryo transfer was performed with a second ultrasound in standing position immediately after transfer,

allowing the movement of embryo-associated air to be assessed. No movement occurred in 94.1% (95/101) of transfers, movement of <1 cm in 4.0% (4/101) of transfers and movement of 1-5 cm in 2.0% (2/101) transfers. No movement of embryo-associated air out of the uterine cavity, either into the cervix or the intramural portion of the Fallopian tube, was seen. The authors summarized that standing shortly after embryo transfer does not play a significant role in the final position of embryo-associated air and is unlikely to be a factor in determining the position of embryos transferred to the uterine cavity during treatment with IVF.

Knutzen et al. (48) demonstrated the potential risk of contrast medium being expelled from the uterine cavity along the path of a transfer catheter. It was unfortunate, however, that the volume of contrast medium injected in this study was substantially more than is usually used. Krampl et al. (27) have demonstrated that the use of embryo transfer-associated air bubbles does not affect pregnancy rates adversely in IVF therapy and has some potential advantages of minimizing capillary action within the narrow diameter catheters used for embryo transfer. All of these studies have the limitation of not actually tracking the movement and position of embryo associated phenomena. They are, however, valuable in adding to our understanding. Transvaginal ultrasound tracking of a transfer-associated air bubble also clearly has limitations, as the embryos themselves are not observed. It would however appear to be an in-vivo direct observational method capable of providing insight into potential embryo movement on standing after transfer. It is impossible, however, to assess whether the embryos moved independently of the intrauterine air bubble. Our laboratory experience has been that on occasions embryos will attach to air bubbles and be moved with them upon manipulation. Embryos may alternatively move in the opposite direction. For example, they may settle to the lower endometrial surface while the air bubble rises. Therefore the direction of movement of the air bubble may not necessarily reflect the direction of movement of embryos. However, it is our opinion that no movement of the air bubble strongly implies that the embryos also remain stationary. These results are consistent with the hypothesis that gravity is unlikely to be a significant force affecting the position of embryos within the uterine cavity following transfer. It needs to be appreciated that the so-called endometrial cavity is a potential and not a real space. Upon insertion of embryo transfer catheters the endometrial surfaces are separated and then re-oppose once the catheter is removed. The embryos, fluid and air injected into this potential space are then subjected to the contractile forces of the myometrium and endomyometrial peri-

stalsis (49-50). Indeed, authors (15, 51) have recently demonstrated the considerable potential of endomyometrial peristalsis to have an effect on the outcomes of patients with infertility and particularly those being treated with IVF. It would seem likely that these forces along with surface tension generated by the fluid/solid interface are likely to be far more potent in determining the final position of transferred embryos.

Baba et al. conducted a prospective analysis to investigate where human embryos implant after ET using transabdominal and transvaginal three-dimensional ultrasound examinations. The main outcome measures of this study were the location of ET-associated air bubbles in the uterine cavity and the location of the resultant gestational sac. Sixty ETs resulted in 22 pregnancies, and 32 gestational sacs were located. Twenty-six of the 32 embryos were within or between the area in which the catheter tip was situated and the area over which air bubbles had spread immediately after ET. The authors concluded that in cases of pregnancy achieved through ET, approximately 80% of embryos implant in areas to which they initially are transferred and approximately 20% implant in other areas (23). It was also our own observation that the embryos don't seem to retain their place of deposition at the time of ET. We observed a transient motion of the ET associated air-bubbles and performed a prospective study to determine if this air-bubble movement at the time of ETs can predict a successful outcome. Trans-abdominal ultrasound guided embryo transfers were performed in 123 consecutive donor egg recipients receiving identical hormonal replacement therapy. All ETs were done by the same physician using the same ET catheter (1816N, Wallace, UK) and the same Culture Media volume (40-50 micro liters). Observation was made of the embryo transfer catheter placement in relation to the endometrial surface and uterine fundus during embryo transfer. Ultrasound-guided tracking of embryo-associated air within the uterine cavity was done immediately after the piston was depressed at the time of embryo deposition. The air-bubble either moved up or down. The Endometrial thickness, the number of embryos transferred and the number of Grade A embryos was not significantly different in the two arms. The air-bubble movement was upwards (Group A) towards the fundus in 66 ETs compared to a downward movement (Group B) in 57 transfers. No movement of embryo-associated air out of the uterine cavity, either into the cervix or the intramural portion of the Fallopian tube, was seen. The Clinical Pregnancy / Embryo Transfer was similar in both Groups; 50% in Group A versus 49.12% in Group B. There was surprisingly one ectopic pregnancy seen in Group B. The Multiple gestation

rate/embryo transfer was 15.15% in Group A versus 15.52% in Group B. The Total Implantation Rate/Embryo Transferred was 22.22% in Group A compared to 19.50% in Group B. The movement of the ET associated air-bubble or the final position of embryo-associated air is unlikely to be a factor in predicting success in donor egg IVF recipient treatment cycles (50). This study also suggests that the ectopic pregnancy incidence is unrelated to the air-bubble movement.

Trial transfer

There is a consensus in the IVF community that a smooth embryo transfer is critical for achieving high success rates (4, 5, 12, 52, 53). To this end, a trial embryo transfer is commonly performed on patients at some point prior to the actual embryo transfer. The 'dummy' trial, or 'mock' transfer can be done before the stimulation cycle, or even right before the actual embryo transfer (54). This procedure is important to evaluate the length and direction of the uterine cavity and cervical canal and to choose the most suitable catheter for the embryo transfer. It also helps to discover any unanticipated difficulty in entering the uterine cavity, such as pin-point external os, the presence of cervical polyps or fibroids, and anatomical distortion of the cervix from previous surgery or due to congenital anomalies. If cervical stenosis is diagnosed, it is advisable to perform cervical dilatation before ovarian stimulation. Another way of evaluating the uterine cavity is by using ultrasonography (US). It gives precise information about the length of the uterine cavity, the length of the cervical canal and a description of cervical angulations in relation to the uterine cavity. It is also very important for diagnosing any fibroids that may be encroaching on the uterine cavity or distorting the cervical canal. Revising the US picture of the uterine cavity right before embryo transfer resembles reading a map or a guide before performing the transfer, which is essentially a blind technique. Many practitioners advocate the use of transabdominal ultrasound guidance during embryo transfer (17,22,55-57); however, this practice is not uniformly employed. In a survey by Kovacs (52) about the relative importance of different factors affecting the success of embryo transfer; ultrasound guidance was ranked (11) out of (12) possible factors. In our program, we have routinely conducted mock embryo transfer just before the actual embryo transfer. For the last several years, the mock embryo transfer and the actual embryo transfer have been both performed under transabdominal ultrasound guidance.

Pope et al. investigated the influence of transfer distance from the fundus (TDF) on clinical pre-

gnancy rate (PR) and ectopic pregnancy rate on 699 ultrasound (US)-guided embryo transfers. Mock transfer was performed to measure uterine cavity depth 1 month before treatment. Cavity depth was measured by abdominal US before the transfer, from the vaginal stripe to the fundus. Transfers were performed with a Wallace embryo transfer catheter using US and physician's judgment of cavity depth. Transfer distance from the fundus was calculated by subtracting the depth of catheter insertion from the cavity depth, as determined by US or by mock transfer. The authors observed that the TDF by US was highly predictive of PR; TDF by mock was not predictive of PR.

Increasing the TDF by US resulted in significantly increased PR as well as lower ectopic rater. Using regression analysis, the odds ratio for TDF by US was 1.11 (95% CI: 1.07-1.14). This suggests that for every additional millimeter embryos are deposited away from the fundus, the odds of clinical pregnancy increased by 11% (56).

Henne & Milki perform a mock USG guided ET remote from the real ET (55). Their data suggest that when the uterus is AV at mock embryo transfer, it is very likely to remain so at the time of actual fresh embryo transfer (98%). Slightly more than one quarter of their patients were found to have an RV uterus at mock embryo transfer. In these patients, there was a significant chance that the uterus would become AV at the time of actual embryo transfer (55%) in fresh IVF cycles. For thawed embryo transfer cycles, the same pattern was noted, where significantly more RV uteri became AV compared with the reverse. The difference, although statistically significant, was not as pronounced. This is consistent with the expectation that the enlarged ovaries lying in the posterior cul-de-sac are a factor in fresh embryo transfer cycles, where controlled ovarian hyperstimulation (COH) is used, and not a factor in thawed embryo transfer, when the cycle is natural. In view of the enlarged ovaries resulting from COH, it is expected that more AV uteri would remain AV at the time of fresh embryo transfer (98%) compared with thawed embryo transfer (88%) performed in a natural cycle. For patients with an RV uterus at mock embryo transfer, more converted to AV at fresh embryo transfer (55%) than at thawed embryo transfer (33%). In this group, there was a significantly higher number of oocytes recovered in fresh cycles when the uterus changed from RV to AV (11.0 v/s 6.4) compared with when the uterus remained RV (8.8 v/s 5.8) (P = 0.01), again confirming the role of the ovarian size in changing the uterine position. However, the fact that the conversion of the uterus from RV to AV was still seen in one-third of the thaw cycles suggests that the ovarian volume is not the only factor that may play a role in uterine position change. Both the mock and real embryo transfers were performed under transabdominal ultrasound guidance, with a full bladder for adequate ultrasound visualization. We are, however, more likely to require a fuller bladder for the real embryo transfer, where, in addition to visualization, more complete straightening of the cervico-uterine angle is desirable (56, 57). The impact of bladder fullness weighing down on the uterus is more likely to prevent anteversion of the uterine position. Therefore, the effect of the full bladder, if anything, would have decreased the conversion from RV to AV at the time of embryo transfer.

Although transabdominal ultrasound-guided embryo transfer is used by many IVF programs, it is far from being a universal practice. Many physicians feel comfortable that the 'clinical touch' can lead to an adequate placement of the embryos in the uterine cavity. It can be argued that experienced IVF practitioners can feel their way along the cervical and endometrial canal while threading the embryo transfer catheter, and thus, it may not be critical for them to visualize the uterine position during embryo transfer. However, based only on clinical touch, many clinicians may be unaware that mal-positioning of the catheter is occurring (15, 16, 56). A gentle direction of the catheter following the contour of the endometrial cavity is essential to avoid disrupting the endometrium and eliciting deleterious uterine contractions, which may expel an embryo (2, 58). An accurate knowledge of the uterine angle at the time of embryo transfer will help with a smooth single motion passage of the embryo transfer catheter. Assuming that an RV uterus at mock embryo transfer will remain RV at actual embryo transfer may initially mislead the practitioner performing the procedure. The realization of a change in uterine position may come only after some hesitation and subtle trauma to the uterus or risk of plugging the catheter tip with endometrium (56, 59). This hesitation may lead to the unnecessary application of a tenaculum to straighten the uterine angle, which may also induce harmful uterine contractions (2, 57). The orientation of the cervix in the vaginal vault may provide an indication of the uterine position in many instances; however, this may not always be accurate. Ultrasonographic guidance during embryo transfer offers the benefits of catheter visualization to confirm passage beyond the internal os and avoid touching the uterine fundus (4, 15, 22). In addition, the lack of consistency between uterine position at mock and actual embryo transfer for patients with RV uteri further supports the use of transabdominal ultrasound guidance in order to more accurately assess the cervico-uterine angle at the time of embryo transfer and gently guide the catheter into the endometrial cavity.

From a practical standpoint, even for programs

that routinely practice transabdominal ultrasound-guided embryo transfer, knowing that an RV uterus will more often than not become AV at embryo transfer supports asking all patients to present for embryo transfer with a full bladder. It has been our experience that patients with a known RV uterus are often instructed to have an empty bladder, or possibly a minimally full bladder, to provide a 'sonic window' for ultrasound visualization, during embryo transfer. For those whose uterus is pushed to an AV position by the enlarged ovaries in the posterior cul-de-sac, a substantially fuller bladder is desirable for a smooth transfer. Presenting with an empty bladder may lead to a suboptimal transfer or entail a significant delay in performing the embryo transfer at the scheduled time. Even for patients with an RV uterus undergoing a frozen thawed embryo transfer, the recommendation for a full bladder is likely to be beneficial, since one-third of these will convert to an AV position.

Other Alternatives to Ultrasound Guidance!

Yanushpolsky et al. evaluated a new technique designed to improve access to the endometrial cavity through tortuous and/or stenotic endocervical canals in women with histories of difficult IUIs, ETs, or endometrial biopsies (60). Women with histories of difficult intrauterine procedures because of tortuous and/or stenotic endocervical canals who continued to undergo treatment had an hysteroscopic evaluation and/or correction of the endocervix, followed by transcervical placement of a Malecot catheter (CR Bard Inc., Covington, GA) for an average of 10 days. Thirty-two of 36 patients had significantly easier procedures after the placement and removal of a Malecot catheter. The authors concluded that hysteroscopic evaluation and placement of a Malecot catheter is a useful technique that allows easier entry through the cervical canal in patients in whom previous IUIs, ETs, and endometrial biopsies have been difficult (60). This procedure may lead to improved pregnancy rates, particularly with IVF-ET, as the ease of ET has been correlated with improved implantation rates.

Discussion

Ever since the birth of the first in-vitro fertilization (IVF) baby in 1978 (61), the advancement in ovulation stimulation regimes, oocyte collection and culture mediums has been phenomenal. However, the technique of uterine embryo transfer remains largely unchanged, since it was first described. Conversely, although there have been vast improvements with ovulation induction, fertilization and embryo cleavage, the majority of transferred embryos fail to implant. This failure may be ascribed to deficiencies in either intrinsic embryo quality or uterine receptivity as suggested by Speirs (62), or it could also logically be due to the technique of embryo transfer. Another more obscure factor affecting embryo implantation may include uterine contractions. Fanchin et al. (58) noted that more uterine contractions at the time of embryo transfer were associated with a lower clinical and ongoing pregnancy rate. The technique of embryo transfer in the majority of the centers world-wide relies on the clinical touch in positioning the transfer catheter in the upper part of the uterine cavity. It would appear that any assistance such as ultrasound guidance in ensuring that the embryos are indeed placed in this position would be desirable. Attempts are constantly being made to improve clinical pregnancy rates after IVF and embryo transfer. Kojima et al. retrospectively examined the efficacy of transvaginal ultrasound guidance during embryo transfer on pregnancy and implantation rates. The results of 846 cycles from their IVF-embryo transfer programme were analyzed and comparisons were made between those carried out using ultrasound guidance and those by the clinical touch method. Higher pregnancy and implantation rates (28.9 and 15.2% respectively) were found in the group using the transvaginal ultrasound guidance during embryo transfer compared with those in the group using the clinical touch method (13.1 and 7.0% respectively). The differences were statistically significant ($P < 0.01$). There was no significant difference in ectopic pregnancy rates between the two groups. The authors concluded that the use of transvaginal ultrasound-guided embryo transfer significantly improved both pregnancy and implantation rates. Although technically difficult, Kojima et al. suggest its use may maximize the chances of achieving a successful pregnancy outcome (63).

A prospective randomized controlled trial was performed to compare embryo transfer under ultrasound guidance versus the clinical touch method (64). A total of 800 embryo transfers was studied; 400 were randomized to ultrasound-guided transfers and 400 were randomized to the clinical touch group. Of these, 441 were fresh cycles and 359 were frozen-thawed cycles. The clinical pregnancy rate was 26.0% in the ultrasound-guided group and 22.5% in the clinical touch group; the difference was not statistically significant. The ongoing pregnancy rate was 23.5% in the ultrasound-guided group compared with 19.0% in the clinical touch group and the difference was again not statistically significant. The implantation rate was slightly higher in the ultrasound-guided group (15.3%) than the clinical touch group (12.0%) (P =

0.048). There were no differences in the incidences of ectopic pregnancy, miscarriage and multiple pregnancy between the two groups. The authors concluded that a significant improvement in implantation rate was observed following the use of ultrasound guidance during embryo transfer. The extent of improvement in the pregnancy rate may depend on the specific techniques and methods of embryo transfer used in individual centers (64).

The use of ultrasound-guided embryo transfer has been reported to affect success rates in some centers but not others. In a prospective study, Prapas et al. examined the influence of ultrasound guidance in embryo transfer performed on different days after oocyte retrieval (65). Two different methods of embryo transfer were evaluated in 1069 consecutive transfers. The ultrasound-guided embryo transfer was used in 433 cases, whereas 636 embryo transfers were performed with the tactile assessment ('clinical feel') method. Ultrasound-guided embryo transfer yielded a higher overall pregnancy rate than the 'clinical feel' approach, 47 versus 36% (P < 0.001). This difference was statistically significant where embryos were transferred after 3 or 4 days of culture, 45.9 versus 37.1% (P = 0.001) and 42.3 versus 27% (P = 0.035) respectively but not significant (P = 0.112) on day 5 embryo transfer (56.3 versus 45.7%). Likewise, the implantation rate was significantly different between the two groups on day 3 and 4 embryo transfer, 23.3 versus 15.8% (P < 0.01) and 21.6 versus 15.7% (P < 0.05%) respectively but no statistical difference was noted on day 5 embryo transfer, 26.7 versus 23.6%. Ultrasound assistance in embryo transfer on day 3 and 4 significantly improved pregnancy rates in IVF but had no impact on day 5 (65).

Two changes in technique of embryo transfer of potential clinical importance were evaluated over two contiguous time periods in order to observe any corresponding change in clinical pregnancy (CP) rate per transfer: (i) embryo transfer catheter; (ii) ultrasound guidance. Catheter choices were hard: Tefcat, Tom Cat, or Norfolk; or soft: Frydman or Wallace. Ultrasound visualization was considered to be excellent/good when the catheter could be followed from the cervix to the fundus by transabdominal ultrasound with retention of the embryo-containing fluid droplet; fair/poor if visualization could not document the sequence of events. Embryo transfers were performed in 518 cycles. CP rates per transfer using soft and hard catheters was 36 and 17% (P < 0.000) respectively. CP rates per transfer for transfers performed with and without ultrasound guidance were 38 and 25% (P < 0.002) respectively. A statistically significant difference was also noted when visualization ranks were compared. CP rates per transfer in all excellent/good ultrasound-guided transfers was 41.5

versus 16.7% for fair/poor transfers (P < 0.038). In conclusion, performance of embryo transfer with a soft catheter under ultrasound guidance with good visualization resulted in a significant increase in clinical pregnancy rates (22).

Using the ovum donation model to eliminate confounding variables, Lindheim et al. assessed the impact of US guided ET on pregnancy rates, implantation rates, and multiple gestation rates. All women who underwent IVF-ET cycles using donated oocytes from November 1997 to September 1998 (n = 137) were evaluated retrospectively. ET from November 1997 to April 1998 were performed without US, while all ET from May 1998 to September 1998 were performed using transvaginal or transabdominal US. ET was further categorized as easy or difficult. Difficult ET was defined as requiring at least two attempts and/or the presence of blood on the catheter and/or > 5 min. Pregnant patients (n = 73) were similar with respect to the number and morphology of the embryos transferred compared to non-pregnant patients (n = 65). US guidance significantly improved implantation and pregnancy rates in cycles with easy transfers [28.8 vs. 18.4% and 63.1 vs. 36.1%, respectively (P < 0.05)] without impacting multiple pregnancy rates. The authors concluded that US guided ET is simple and reassuring and appears to significantly improve pregnancy outcomes in ovum donation cycles by optimizing the placement of embryos (19).

Sallam and Sadek conducted a meta-analysis of randomized controlled studies to evaluate abdominal ultrasound-guided embryo transfer compared to the clinical touch method (8). Out of a total of 2,051 patients: 1,024 received ultrasound-guided embryo transfers and 1,027 received clinical touch method transfers. The authors observed that compared to the clinical touch method, abdominal ultrasound-guided transfer significantly increased the clinical pregnancy rate and the ongoing pregnancy rate. There was no effect on the incidence of ectopic pregnancy, multiple pregnancy, or miscarriage rate (8).

Buckett performed a systematic review and meta-analysis of randomized, controlled trials comparing ultrasound-guided embryo transfer with embryo transfer by clinical touch alone. Meta-analysis demonstrated a significantly increased chance of clinical pregnancy following ultrasound-guided embryo transfer in all studies and in the genuinely randomized subgroup. The embryo implantation rate was also significantly increased following ultrasound-guided embryo transfer (6).

A retrospective analysis of 823 consecutive embryo transfers was performed by Mirkin et al. to determine the impact of transabdominal ultrasound guidance on embryo transfer during IVF therapy (9).

Three hundred and sixty-seven procedures performed with transabdominal ultrasound guidance were compared to 456 cases performed with the "clinical touch" method. Ultrasound-guided embryo transfer yielded higher, but not statistically significant, clinical pregnancy (48% vs. 44%) and implantation rates (22% vs. 20%). The incidence of multiple pregnancies, ectopic and multiple pregnancy rates were similar. The frequency of negative factors typically associated with difficult transfers, such as requirement of use of tenaculum, and presence of blood or mucus in the catheter tip, were significantly lower in the ultrasound-guided group in comparison with the clinical touch group. Ultrasound-guided embryo transfer was associated with a significantly increased easiness of transfer performance; 95% of the transfers were rated as very easy in the ultrasound guidance group compared to 87% in the clinical touch group. The use of a soft pass catheter was the only variable independently and significantly associated with pregnancy success (odds ratio = 2.74). The study concluded that ultrasound-guidance facilitates embryo transfer and in combination with the use of a soft catheter should be implemented to optimize embryo transfer results (9).

Garcia-Velasco et al. conducted a prospective, randomized, controlled trial to determine whether transabdominal ultrasound guidance during embryo transfer (ET) is a useful tool for increasing pregnancy rates in patients undergoing oocyte donation (66). Clear visualization at ultrasound during ET was achieved in 90.8% of the patients who had ultrasound-guided ET. A similar number of easy transfers were performed in both the ultrasound-guided and the control groups (84.5% vs. 86.6%). The pregnancy rate was comparable between the groups (59.9% ultrasound vs. 55.1% control), as was the implantation rate (30.6% ultrasound vs. 26.3% control). No differences were found in the miscarriage rate (10.7% ultrasound vs. 9.1% control) or in the multiple pregnancy rate (21.4% ultrasound vs. 22.5% control). Although all ectopic pregnancies occurred in the group that did not receive ultrasound guidance, the differences were not statistically significant (0 vs. 2.7%). They authors could not show any benefit in terms of pregnancy rate in oocyte recipients for whom ET was performed under direct transabdominal ultrasound visualization of the endometrial cavity. There was a lower ectopic pregnancy rate when ultrasound guidance was used, but this rate was not statistically significant in comparison with the pregnancy rate without ultrasound guidance (66).

Sharif et al. (54) have published a non-randomized clinical experience of 103 cases in which the patient was asked to stand shortly after embryo transfer without apparent detriment to pregnancy rates. Al-Shawaf et al. (67) attempted to address the issue of whether technical factors such as transfer catheter or ultrasound assistance had any impact on IVF treatment cycle outcome. In their study, patients were also asked to stand straight after transfer. The reported pregnancy and implantation rates in both studies remained high. Many patients in the past have expressed their concern about the possibility of embryos 'falling' out of the uterus on standing after their transfer. This is particularly so for those long term patients who had experienced previous requests to lie down for some time after embryo transfer. Progressively increasing pregnancy rates at the same time as reducing the length of time patients are allowed to lie down after embryo transfer gives us reassurance that standing up quickly is unlikely to be a factor which significantly affects pregnancy rates.

Searching on Medline revealed that the number of scientific publications on human IVF from the years 1978-2003 is 50,200. However, the number of scientific publications on the technique of embryo transfer is only 65. That discrepancy reflects how little attention has been given to the technique of embryo transfer. It is estimated that poor embryo transfer technique may account for as much as 30% of all failures in assisted reproduction (68). Unfortunately, this failure must have affected thousands of couples every year since the beginning of IVF. This final step in assisted reproduction will determine the fate of a long period and a lot of effort, from ovulation induction and ovum retrieval, to the tedious high technology procedures in the laboratory.

Allowing the initiation of any uterine contractility leads to immediate or delayed expulsion of the embryos after transferring them into the uterine cavity and this has always been of concern in assisted reproduction. The presence of endometrial movements has been recognized by several groups (69-71). About 15% of transferred embryos have been collected from the external cervical os, the tip of the catheter and vaginal speculum after embryo transfer (72). Menezo et al. (73) were able to demonstrate that only 45% of embryos were present within the uterine cavity 1 hour after the transfer. Stimulation of the cervix causes the release of oxytocin, thus increasing uterine contractility. In a prospective clinical study serial blood samples were collected in time intervals of 20 seconds during the embryo transfer procedure in order to measure serum oxytocin concentration (74). It was found that in the absence of tenaculum placement, no increase in serum oxytocin concentration was observed. When tenaculum was used, it was temporarily associated with an elevation in oxytocin level, which remained elevated until the end of the embryo transfer procedure. Injection of oxytocin induces uterine contractions at all stages of the estrous cycle in the cow (75). In an early study on

cows, 'artificial embryos' consisting of resin spheres impregnated with radioactive gold were used. It was found that after 1.5 hours, a large proportion of the spheres had been expelled from the uterus altogether (76). In a study on humans by Knutzen et al. (48) using radio-opaque dye, mimicking embryo transfer, it was found that the dye remained primarily in the uterine cavity in only 58% of cases, and it was concluded that the remainder of the patients would have lost their opportunity for pregnancy as a result of the embryo transfer procedure (48).

Several precautions can be taken to avoid the initiation of uterine contractions. The ideal embryo transfer catheter should be soft enough to avoid any trauma to the endocervix or endometrium and malleable enough to find its way into the uterine cavity. Since the very early days of IVF, the value of soft embryo transfer catheters has been recognized. Several studies have compared different kinds of catheters for embryo transfer and have demonstrated that soft catheters are the best in terms of pregnancy rates (22, 68, 77, 78, 79). In a study of 518 IVF cycles, the clinical pregnancy rates per transfer using soft and bard catheters were 36 and 17% respectively (22), although other studies have found no difference in the pregnancy rate with respect to the catheter used (80). The word 'soft' means a combination of physical flexibility, malleability and smoothness of the tip (68). It is important to mention that in order to benefit from the advantages of the softness of the catheter, the outer rigid sheath should be minimally used just to stop short of the internal os and never touch the internal cervical os. The stimulus of the transfer catheter passing through the internal cervical os can also initiate contractions, which are probably mediated by the release of prostaglandins (81). That is why, in the human, it is advisable to perform the embryo transfer without manipulation of the cervix (3, 74).

It is essential to be absolutely sure that the embryo transfer catheter has passed the internal os and entered the uterine cavity. Soft catheters can sometimes be misleading, as they can curve inside the cervical canal. Experienced practitioners can discover this easily. A simple test that can be done to insure that the soft catheter has passed the internal os and not simply bent inside the cervical canal is to rotate the catheter 360°. If it recoils, it means that it is curved inside the cervical canal. One important cause for the failure of the catheter to pass the internal os is simply a lack of alignment between the catheter (straight) and the utero-cervical canal (curved or angulated). A simple procedure of gently curving the outer sheath of the catheter will overcome this problem in most cases. Ideally, a situation in which you have the embryos loaded and you need to make a curve in the catheter should be completely avoided. Proper eva-

luation of the utero-cervical axis and determining how much curvature is needed for the catheter should be done before loading the embryos. Performing a dummy embryo transfer right before the actual one and revising the previously performed US picture of the uterus can easily achieve this. Straightening the uterocervical angle can be achieved by a full bladder before embryo transfer. This effect is being achieved indirectly by performing embryo transfer under US guidance in some centers. Another simple way to facilitate entering the catheter is by gently maneuvering the vaginal speculum (the degree of opening and how far it is pushed inside). In some cases you need to use a more rigid catheter so that it can pass the internal os. It is essential that these rigid catheters are malleable. Malleability is important to allow the making of a curved shape, which facilitates the introduction of the catheter inside the cavity. This will overcome acute angulations. In rare cases the cervix has to be held by a volsellum in order to stabilize the uterus while introducing the catheter. The effect of cervical traction with a tenaculum on the utero-cervical angle was studied using radio-opaque guidewire (82). It was found that moderate cervical traction straightens the uterus and it was concluded that the routine use of the tenaculum theoretically makes the passage of an embryo transfer catheter easier and less traumatic. However, one should not forget that holding the cervix with a volsellum leads to the release of oxytocin (74). In difficult procedures, embryos were found to be retained in the embryo transfer catheter significantly more often than in easy transfers (59). However, the authors found that the pregnancy rate was not compromised when the retained embryos were discovered and immediately retransferred into the uterine cavity. One possible reason for retained embryos is the position of the embryos in the catheter. Small volumes of <40 microliters are preferable, but it is important that 20 microliters of fluid is aspirated first, and then the embryos are aspirated second. This will ensure enough media to push out the embryos; in the mean time, once injection is done it is advisable to keep the pressure on the plunger of the syringe until withdrawal of the catheter (12). Another important precaution to minimize retained embryos in the catheter is a slow withdrawal of the catheter after injecting the embryos. Rapid withdrawal may create a negative pressure and result in the withdrawal of the embryos following the catheter.

Embryo transfer is occasionally difficult in women with pronounced uterine flexion, scarring in the lower uterine segment or distorted endometrial cavity (83). For these extremely difficult cases, stiffer and more rigid catheter systems can be used (54, 78). A co-axial catheter system has been used with success in women with a history of difficult or failed embryo

transfer (83). In rare cases, transmyometrial surgical embryo transfer can be used (84, 85). This surgical embryo transfer has been used successfully by some groups, achieving results comparable with the transcervical route (86).

Both the patient and her partner have the opportunity to be involved and directly visualize the transfer of their embryos to the uterine cavity with USG guidance. It allows their involvement and commentary on the process and the psychological security of the satisfactory completion of the technical components of their treatment cycle.

It has been our experience as a result of over 1000 ultrasound-guided embryo transfers that it is extremely difficult and usually impossible to reposition the outer guiding catheter to avoid a posterior path of the embryo transfer catheter toward or into the endometrium. We believe we were able to differentiate between those cases where the catheter indented the endometrium and those in which the catheter had penetrated the endometrial surface and embedded beneath the surface leading to intra-endometrial embryo transfers. With catheter indentation of the endometrium, the embryo-associated air was seen to be positioned after transfer between the anterior and posterior endometrial surfaces at their visible junction as identified by a central endometrial echo; however, with intra-endometrial transfer the hyper-echoic area of the air was seen to be positioned beneath the central endometrial echo. Whether intra-endometrial embryo transfer will be demonstrated to have any effect on pregnancy rates is debatable, although it would seem reasonable to adopt a practice of performing embryo transfer in the least traumatic method possible. On the other hand, Kato et al. (84) have suggested that transvaginal transmyometrial embryo transfer may not only have advantages for those patients with severe cervical stenosis where transcervical transfer is impossible but may also in itself be beneficial in permitting intra-endometrial embryo transfers. This proposition has not been supported by follow-up studies by Kato or others but nevertheless indicates that pregnancy is possible following what might appear to be a relatively traumatic transfer methodology.

Endometrial Cavity Fluid

Fluid within the endometrial cavity before embryo

transfer in IVF cycles is associated with failure of implantation. The etiology of endometrial fluid is surrounded in controversy but it is associated with hydrosalpinges, polycystic ovarian disease, and subclinical uterine infections. The current treatment consists of postponing embryo transfer. This of course has biological and psychological disadvantages; a decreased implantation rate from frozen embryo transfer, and frustration and disappointment for the couple. Removing the fluid with an embryo transfer catheter immediately before embryo transfer may be a successful method of treatment (87).

Conclusions

Since the beginning of the therapeutical application of IVF, many programs have followed in an empirical, but traditional, manner the method of 'clinical touch'. This technique consists of the insertion of a catheter into the cavity until touching the fundal endometrium, followed by a 5/10 mm retreat and subsequent deposition of the embryos.

This method was first described by Steptoe and Edwards but, despite being the best known technique for embryo transfer, its difficulties and uncertainties have been widely questioned. One of these uncertainties is related to the fact that transfers based only on the sensitivity of the operator are associated with discrepancies between the presumed and true position of the catheter, especially considering the different levels of clinical experience (6-8, 15, 17).

On the other hand, ultrasound-guided embryo transfer ensures the exact position of the catheter in the uterine cavity (and, consequently, the site where the embryos will be transferred and probably implant), in addition to preventing touching the fundal area and thus the occurrence of bleeding and uterine contractions (6-9, 20-23).

Although there are no absolute prerequisites, ultrasound monitoring to ensure correct catheter placement, pretreatment assessment of the cervical canal, minimization of trauma, avoidance of mucus and the use of a 'soft' catheter are all considered important factors in this process. Early patient mobilization after the transfer procedure has not been shown to influence the outcome. The establishment of a benchmark against which to evaluate individual performance and participation in 'refresher courses' if indicated will help to maximize the implantation rates of the assisted reproductive technology programme as a whole (88).

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