

## OPINION

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### Optimizing the embryo transfer technique

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**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. Finally, one has to be absolutely sure that the embryo transfer catheter has passed the internal cervical os and that the embryos are delivered gently inside the uterine cavity.**

*Key words:* catheters/embryo transfer/IVF/ultrasonography evaluation/uterine contractility

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#### Introduction

Embryo transfer is the final and most crucial step in IVF. About 80% of patients undergoing IVF reach the embryo transfer stage (Human Fertilisation and Embryology Authority, 1996), but only a small portion of them achieve pregnancy. The pregnancy rate after embryo transfer is dependent upon multiple factors including embryo quality, endometrial receptivity and the technique of the embryo transfer itself. This article will focus only on the technique of embryo transfer, mainly the transcervical route. Embryo transfer technique has been given little attention and the published data on the subject are minimal. Searching on Medline revealed that the number of scientific publications on human IVF from the years 1978–2001 is 40 500. However, the number of scientific publications on the technique of embryo transfer is only 45. That discrepancy reflects how little attention has been given to the technique of embryo transfer. And the reason for that is due to the apparent simplicity of the embryo transfer technique. To most clinicians, it is not a difficult task to insert the embryo transfer catheter and eject the embryos. Unfortunately, it is not as simple as it looks and is easier said than done (Naaktgeboren *et al.*, 1998).

The importance of the technique by which the embryos are transferred is reflected in the difference in the pregnancy rates associated with different individuals performing the embryo transfer within the same programme. In previous studies (Karande *et al.*, 1999; Hearn-Stokes *et al.*, 2000), significant differences were observed in pregnancy rates after the embryo transfer was performed by different providers, suggesting that the embryo transfer technique may directly influence the

outcome in assisted reproductive technology. It is estimated that poor embryo transfer technique may account for as much as 30% of all failures in assisted reproduction (Cohen, 1998). Unfortunately, this failure must have affected thousands of couples every year since the beginning of IVF.

The aim of this paper is to review the published data on the technique of embryo transfer and add our own view based on our experience in an attempt to optimize the embryo transfer technique.

#### Suggestions for optimizing the embryos transfer technique

Embryo transfer is routinely carried out using the transcervical route, which is basically a blind technique, associated with multiple potential negative factors that can result in total failure of the whole procedure. These potential negative factors include: (i) initiation of uterine contractility that may lead to an immediate or delayed expulsion of the embryos; (ii) the presence of cervical mucus that can plug the tip of the catheter or entangle the embryos and drag them out during withdrawal of the catheter; (iii) proper placement of the embryos into the uterine cavity may not be achieved due to failure to pass the catheter through the internal os. This can be due to acute utero–cervical angulation, cervical stenosis or anatomical distortion of the cervical canal.

Therefore, extra care and time should be given to embryo transfer, which is the most critical step in 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. The following are some suggestions for optimizing the embryo transfer technique.

### ***Proper evaluation of the uterine cavity***

It is important to perform this step before the IVF cycle in order to ensure the proper placement of the embryos. It has been demonstrated by our group (Mansour *et al.*, 1990) that performing a dummy embryo transfer before the IVF cycle significantly improves the pregnancy rate. The 'dummy' trial, or 'mock' transfer can be done before the stimulation cycle, or even right before the actual embryo transfer (Sharif *et al.*, 1995). 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 polypi 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.

### ***Avoiding the initiation of any uterine contractility***

Immediate or delayed expulsion of the embryos after transferring them into the uterine cavity has always been of concern in assisted reproduction. The presence of endometrial movements has been recognized by several groups (Birnholtz, 1984; Ijland *et al.*, 1996; Kunz and Leyendecker, 1996). About 15% of transferred embryos have been collected from the external cervical os, the tip of the catheter and vaginal speculum after embryo transfer (Poindexter *et al.*, 1986). Ménézo *et al.* were able to demonstrate that only 45% of embryos were present within the uterine cavity 1 h after the transfer (Ménézo *et al.*, 1985).

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 s during the embryo transfer procedure in order to measure serum oxytocin concentration (Dorn *et al.*, 1999). 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 (Harper *et al.*, 1961a). 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 h, a large proportion of the spheres had been expelled from the uterus

altogether (Harper *et al.*, 1961b). In a study on humans by Knutzen *et al.* 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 (Knutzen *et al.*, 1992). In another study conducted by our group (Mansour *et al.*, 1994) using Methylene Blue, it was demonstrated that the dye was visualized at the external cervical os in 42% of the cases. This means that the uterus extruded the dye, at least partially, which was transferred as in the actual embryo transfer. Consequently, it is possible that the embryos may be extruded partially or totally after embryo transfer (Poindexter *et al.*, 1986; Schulman, 1986; Mansour *et al.*, 1994). It has been observed that, after embryo transfer, the embryos can move as easily toward the cervical canal as toward the Fallopian tube (Woolcott and Stanger, 1997).

Several precautions can be taken to avoid the initiation of uterine contractions.

### ***The use of soft catheters***

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 (Wisanto *et al.*, 1989; Mansour *et al.*, 1994; Cohen, 1998; Ghazzawi *et al.*, 1999; Wood *et al.*, 2000). In a study of 518 IVF cycles, the clinical pregnancy rates per transfer using soft and hard catheters were 36 and 17% respectively (Wood *et al.*, 2000), although other studies have found no difference in the pregnancy rate with respect to the catheter used (Diedrich *et al.*, 1989). The word 'soft' means a combination of physical flexibility, malleability and smoothness of the tip (Cohen, 1998). 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 (Fraser, 1992). That is why, in the human, it is advisable to perform the embryo transfer without manipulation of the cervix (Dorn *et al.*, 1999; Lesny *et al.*, 1999a).

### ***Avoid touching the uterine fundus***

It is a fact that if the tip of the catheter touches the uterine fundus, the patients experience immediate discomfort followed by suprapubic pain or heaviness. This is probably associated with the initiation of uterine contractility. It has also been demonstrated that more uterine contractions at the time of the embryo transfer are correlated with lower clinical pregnancy rates. Depositing the embryos in the mid-fundal area of the uterus was found to be important in improving the pregnancy rate (Waterstone *et al.*, 1991; Rosenlund *et al.*, 1996; Naaktgeboren *et al.*, 1997). They reported a significant increase in the pregnancy rate by changing the position of

the catheter so as to avoid depositing the embryos close to the uterine fundus. Transferring the embryos by replacement at 6 cm without tracing the position of the fundus was found to improve pregnancy rates (Naaktgeboren *et al.*, 1998). Other groups routinely place the catheter ~0.5 cm below the fundus (Diedrich *et al.*, 1989). Furthermore, it was demonstrated by Lesny *et al.* that touching the fundus with the catheter stimulated junctional zone contractions that can reduce the chances of pregnancy (Lesny *et al.*, 1998). Depositing the embryos should be done ~1 cm from the fundus or at least 2 cm beyond the internal cervical os. Therefore, individual measurements of the cervical canal and uterine cavity are extremely important for the outcome.

#### *Gentle manipulation*

It has been shown that stimulation of the cervix causes the release of oxytocin, thus increasing uterine contractions (Dorn *et al.*, 1999). Therefore, holding the cervix by a volsellum should be completely avoided except in rare cases. It has been observed that the use of tissue forceps to hold the cervix can trigger uterine contractions (Lesny *et al.*, 1999a). Gentle manipulation should be the rule, even in introducing the speculum to avoid unnecessary pushing of the cervix. The use of soft catheters should be the first choice except in rare cases when it cannot be introduced. In an excellent study in 1998, Lesny *et al.* assessed whether the embryo transfer can alter junctional zone contractility (Lesny *et al.*, 1998). They studied the effect of easy and difficult mock transfers, using an Echovist bolus to represent embryos. They demonstrated that touching the fundus started strong random waves in the fundal area and from the fundus to the cervix, which relocated the Echovist in six out of seven patients. The above findings correlate with other studies that have shown that technically difficult embryo transfers are associated with reduced pregnancy rates (Visser *et al.*, 1993; Sharif *et al.*, 1995; Lesny *et al.*, 1999b). This is probably due to the stimulation of uterine contractions that would expel the embryos (Fanchin *et al.*, 1998). As a general rule, embryo transfer should be a simple and painless procedure. The mere presence of the transfer catheter might be one of the factors that can trigger uterine contractions (Lesny *et al.*, 1999b). Some authors suggest that it is preferable to wait for the release of embryos from the catheter or to wait before withdrawal of the catheter so that the uterus can stabilize (Wisanto *et al.*, 1989; Al-Shawaf *et al.*, 1993). Other investigators achieved good results by withdrawing the catheter immediately after the transfer (Zech *et al.*, 1997). Recently, Martinez *et al.* in a prospective randomized study reported no differences in the pregnancy rate between withdrawal of the catheter immediately after embryo deposit or after a 30 s wait (Martinez *et al.*, 2001).

#### *Getting rid of cervical mucus*

Cervical mucus can be a serious obstacle in proper embryo replacement. Cervical mucus can plug the tip of the embryo transfer catheter, causing difficulty in delivering the embryos inside the uterine cavity, especially with such a small volume of culture media to inject with the embryos. Moreover, the embryos can stick to the cervical mucus around the catheter and be dragged outside during the withdrawal of the catheter.

If the mucus is pushed or injected higher in the uterine cavity, it may interfere with implantation. It has been demonstrated by our group that removing the cervical mucus before a Methylene Blue dummy embryo transfer significantly reduced the appearance of the dye at the external os (Mansour *et al.*, 1994). In a retrospective study by Nabi *et al.* analysing 1204 embryo transfer procedures, it was shown that the embryos were much more likely to be retained when the embryo transfer catheter was contaminated with mucus or blood (Nabi *et al.*, 1997).

#### *Proper delivery of the embryos inside the uterine cavity*

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 evaluation of the utero-cervical axis and determining how much curvature is needed for the catheter should be done before loading the embryos. This can be easily achieved by performing a dummy embryo transfer right before the actual one and revising the previously performed US picture of the uterus. Straightening the utero-cervical angle can be achieved by a full bladder before embryo transfer (Lewin *et al.*, 1997). This effect is being achieved indirectly by performing embryo transfer under US guidance in some centres.

Another simple way to facilitate entering the catheter is by gently manoeuvring 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 (Johnson and Bromham, 1991). 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 (Dorn *et al.*, 1999) and it is painful and should be done under general anaesthesia.

In difficult procedures, embryos were found to be retained



in the embryo transfer catheter significantly more often than in easy transfers (Nabi *et al.*, 1997). 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 µl are preferable, but it is important that 20 µl 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 (Hearns-Stokes *et al.*, 2000). 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.

The use of US guidance to facilitate embryo transfer has been described by various IVF programmes (Strickler *et al.*, 1985; Leong *et al.*, 1986) and has proven useful in women with a previously difficult transfer (Kan *et al.*, 1999). The use of US guidance for embryo transfer was found to be simple and reassuring and significantly improved pregnancy rates by optimizing the placement of embryos (Cohen, 1998; Coroleu *et al.*, 2000; Wood *et al.*, 2000). The clinical pregnancy rates per transfer performed with and without US guidance were 38 and 25% respectively (Wood *et al.*, 2000). In a randomized clinical trial, the pregnancy rate was 50% after US guidance versus 33% using clinical touch (Coroleu *et al.*, 2000). However, other studies found no significant difference between US guided and clinical touch embryo transfer (Al-Shawaf, 1993; Kan *et al.*, 1999). It depends on the experience of the clinician providing the embryo transfer.

In extremely rare cases it is very difficult, or even impossible, to pass the catheter inside. This may be due to anatomical distortion of the cervix by previous surgery or fibroids or due to congenital anomaly. Embryo transfer is occasionally difficult in women with pronounced uterine flexion, scarring in the lower uterine segment or distorted endometrial cavity (Patton and Stoelk, 1993). For these extremely difficult cases, stiffer and more rigid catheter systems can be used (Mansour *et al.*, 1990; Sharif *et al.*, 1995). A co-axial catheter system has been used with success in women with a history of difficult or failed embryo transfer (Patton and Stoelk, 1993). In rare cases, transmyometrial surgical embryo transfer can be used (Kato *et al.*, 1993; Groutz *et al.*, 1997). This surgical embryo transfer has been used successfully by some groups, achieving results comparable with the transcervical route (Sharif *et al.*, 1996).

Regarding whether or not the movement of the patient after embryo transfer has an effect on the position of the embryos, Woolcott and Stanger performed US tracking of the embryo immediately after embryo transfer in a standing position (Woolcott and Stanger, 1998). They concluded that standing shortly after embryo transfer does not play a significant role in the final position of the embryos. In a study that had >1000 cycles and a historical cohort-control design (Sharif *et al.*, 1998) the results strongly suggested that bedrest was not necessary following embryo transfer.

In conclusion, the key factors in optimizing the embryo transfer technique are avoidance of uterine contractility, proper evaluation of the uterine cavity, removal of cervical mucus and, finally, ensuring that the catheter has passed the cervical os, delivering the embryos inside the uterine cavity.

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