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Review article

Factors affecting the successful embryo transfer

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Summary

The main factors affecting pregnancy and implantation rates are uterine receptivity, embryo quality, and transfer efficiency. Embryo transfer (ET) is the last step of critically important procedure of in vitro fertilization (IVF) and probably the least successful step in Assisted Reproductive Technology (ART) treatment cascade; though simple in most of the cases, it may pose to be the most difficult in some.
No matter how good the IVF laboratory culture environment is, the physician can ruin everything with a carelessly performed embryo transfer. The entire IVF cycle depends on delicate placement of the embryos at the proper location near the middle of the endometrial cavity.
Pregnancy rates will be significantly increased with the following procedures:
1. Trial transfer
2. Avoiding the initiation of uterine contractility by using soft catheters, gentle manipulation and by avoiding touching the fundus.
3. Removal of cervical mucus, wash and lavage of cervix with culture media.
4. Ultrasound-guided ET with full bladder.
5. Deposition of the embryo 2 cm below the uterine fundus.
6. Examination of catheter following transfer for retained embryos, blood and mucus.

Slow withdrawal of the embryo transfer catheter, the use of a fibrin sealant, bed rest after embryo transfer, sexual intercourse and routine administration of antibiotics following embryo transfer remained to be studied by randomized clinical trials (RCTs).

Key words: *Embryo transfer, In vitro fertilization, Pregnancy rate.*

Introduction

The procedure of embryo transfer is performed by a physician, often with the aid of ultrasound to allow for precise placement. The catheter loaded with one or more embryos is advanced through the cervix into the uterine cavity and the embryo(s) are released into the cavity. Anesthesia is generally not required.

Although unexercised embryo transfer is a significant limiting factor in pregnancy rates after ART treatment, many other factors influence clinical pregnancy as well in the IVF cycle such as: patient selection, ovulation induction method, oocyte retrieval techniques, embryo quality, uterine receptivity and embryo transfer technique. Embryo transfer is the last step and is a critical part in IVF cycle and is thought to be the most inefficient. Historically, much less effort has been placed on assessing or maximizing the efficacy of embryo transfer procedures compared to the other aspect of in vitro fertilization. Given the importance of ET, an understanding of factors that influence success at ET is clearly relevant. However, little attention has been given to the technique of embryo transfer.

Over recent years, this has changed and it is now generally accepted that paying a lot of attention to individual variations in transfer technique can have a positive impact on success rate of IVF. Therefore, extra care and time should be given to embryo transfer, which is the most critical step in IVF.

Based on the latest literature, generally, transfer is carried out about 48-50h after oocyte collection, which is about 44-48h after insemination of oocyte. The embryo is usually at the 2-4 cell stage of cleavage. We have moved towards transferring only two embryos in the majority of women, especially those who are felt to be particularly at risk for

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multiple implantations- i.e. women under the age of 36 and those who were previously fertile.

In the past, patients used to lying down for 12-24 h after transfer but this is no longer in use. Traditionally, embryo transfer after IVF has been performed blindly and Physicians usually underestimated the importance of the embryo transfer (1). The basic technique of transferring embryos to the uterus following IVF recommended by Steptoe was to place the patient in the knee–chest position. Kovacs reported the results of a survey of the directors of all IVF units in Australia and Newzealand. A questionnaire asked them their attitude with respect to 12 factors, which constitute the ET matrix. They were requested to rate each step on a scale of 1-10, where one was irrelevant and 10 was very important. The most highly ranked factor was the removal of hydrosalpinges prior to starting a cycle. Surprisingly the use of ultrasound was almost at the bottom of the list. The use of abdominal scanning was criticized as the bladder needed to be filled and the catheter tip was not easily visualized on the scan. Based on the recent literatures the use of ultrasound guidance is becoming routine (2).

Factors that affect ET success rate

Although many clinicians have extensive personal experience, it is difficult for trainees to understand the anatomical and mechanical process of safe and adequate placing of embryos in the uterine cavity. Ultimately, even with ultrasound help, it is a "blind procedure". The importance of the technique by which the embryo is transferred is reflected in the difference in pregnancy rate associated with different individuals performing the embryo transfer within the same program. In one program with unified treatment protocols, patients can expect varying treatment outcomes with different physicians. Outcomes of IVF vary depending on the treating physician. The pregnancy and implantation rates varied significantly for some physicians, depending on whether they were responsible for the choice of stimulation protocol, supervision of cycle monitoring, or ET in their own or other physicians' patients. Lower than expected pregnancy and implantation rates usually are not caused by poor ET techniques alone, but appear to be disproportionately the consequences of poor cycle stimulation (3-5).

The other factors are discussed bellow;

Trial transfer (mock or dummy)

In the early 1990s studies were first published on the use of a mock or "dummy" ET before the start of an IVF cycle (6). A trial of transfer in a cycle preceds IVF to measure the uterine cavity depth and direction of cervix and uterus, or any stenosis of cervix. In addition it is helpful to place a laminaria prior to plan IVF_ET cycle.

Trial transfer can be carried out in a prior cycle or at the time of retrieval. Full bladder and ultrasound guidance is necessary, although a full bladder can sometimes make a difficult transfer, easy. However, only one of these studies was a randomized controlled trial (RCT) where the authors reported that the pregnancy and implantation rates were significantly higher in the dummy-transfer group compared to the no-dummy transfer group(6).

Most center routinely evaluate uterine cavity by hysterosalpingography or hysteroscopy or hysterosonography (one of these technique) and all are efficient. This is to rule out polyps, fibroids, adhesions and other intrauterine abnormalities. Revising the ultrasonography 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. Proper evaluation of the utero-cervical axis and determination of how much curvature is needed for the catheter, should be done before loading the embryos.

The ultimate goal of a successful embryo transfer is to deliver the embryos atraumatically to the uterine fundus in the location where implantation is maximized.

In most cases the ET procedure is performed blindly without any apparent problems. However, several studies have shown that in some cases it can be difficult or even impossible to perform a transcervical embryo transfer (7,8). In these cases, embryo transfer can be performed by introducing a needle into the uterine cavity through the myometrium, referred to as a transmyometrial embryo transfer (TMET). However, in addition to an impossible or difficult transcervical embryo transfer, it has been suggested that a TMET should be performed for other indications such as `fragile' embryos or multiple implantation failure (9-11). In the Towako method a needle is inserted transmyometrially, guided by transvaginal ultrasound, but the tip of the needle is placed into the outer layer of the endometrium at endometrial–myometrial junction. Three methods of embryo transfer had been described under ultrasound guidance: ultrasound guided uterine transfer, ultrasound guided tubal transfer, ultrasound guided myometrial transfer (12). The question, `Which is the most successful method of embryo transfer in cases of complicating cervical factors?' remains unanswered at present. TMET seems an attractive alternative,

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although the increase in junctional zone contractions, forms a theoretical objection.

In one study embryo transfer was difficult in 29.8% of cases where no trial transfer was performed, compared to no difficult embryo transfer in the trial transfer group. The pregnancy and implantation rate were higher in the trial group (13,6).

Technically difficult ETs due to cervical stenosis are associated with reduced chance of pregnancy after assisted reproductive procedures. Therefore, cervical dilatation has been proposed as a means to overcome difficult embryo transfer, but consistent criteria for patient selection are lacking. In patients with prior difficult embryo transfer, cervical dilatation, 1-3 months before embryo transfer lead to an improved pregnancy rate (14).

Cervical mucus

Given the importance of ET, an understanding of factors that influence success at ET is clearly relevant. Although several studies have evaluated variables which may correlate with a reduction in clinical pregnancy rate (CPR) after ET, there exists a significant inconsistency with respect to the presence of blood or mucus on the catheter, retention of embryos in the transfer catheter, and multiple attempts at ET (aborted ET).

Usually after the exposing cervix, any vaginal and cervical secretions are gently removed with small pled gets of cotton wool moistened in normal saline.

This may also be a source of contamination of the endometrial cavity and the embryo and cervical lavage may reduce the amount of bacteria present in the cervical canal. Even some suggested vigorous cervical lavage technique before embryo transfer. All efforts should be made to avoid difficult embryo transfers provoking bleeding or uterine contractions. Poindexter *et al* showed the presence of embryo in the cervical mucus as well as the vagina and speculum after transfer. That is due to " sling shot" effect, cervical mucus adherent to the embryos may result in embryo expulsion after transfer, and therefore failure of the proper placement of embryos at the time of transfer may occur frequently. Evaluation of only the transfer catheter may result in a significant underestimation of the problem (15).

In one study on catheter contamination, embryos with mucus and/or blood compromise the treatment outcome in IVF only when there was associated retained embryo(s). As increased vigilance in searching for extruded embryos may not be practical, it is suggested that cervical mucus should be routinely aspirated and ET should be performed as automatically as possible (16).

Aflatoonian *et al* (17) in a randomized clinical trial found that cervical mucus aspiration before embryo transfer can increase the pregnancy rate.

Hence, aspiration of the mucus was recommended if copious cervical mucus is present.

The patients in whom ET was classified as "difficult," and failed to conceive with previous ET attempts, after cervical mucus aspiration had significantly easier ET procedures compared with previous attempts and achieved clinical pregnancies. In addition, Hysteroscopic revision of the cervical canal results in easier ET and improves pregnancy rates in patients with cervical stenosis and histories of difficult ET (18).

Embryos are significantly more likely to be retained when the embryo transfer catheter is contaminated with mucus or blood and when the transfer procedure is difficult compared with when it is easy. There is no evidence from any study, to suggest that pregnancy rate is compromised when embryos are retained, provided they are discovered and immediately retransferred into the uterine cavity. Immediate retransfer is more convenient to the patients and reduces the laboratory workload without compromising the treatment outcome (19).

Bed rest following embryo transfer

It was shown that immediate ambulation following the ET procedure has no adverse influence on the ability to conceive (20).

In one study, patients were randomized to rest for either 1 or 24 hours after embryo transfer. One-hour and 24-hour rest post-embryo transfer result in comparable rates of clinical pregnancy. However, 24-hour rest results in reduced implantation rate per embryo (21).

The place of embryo placement

In a RCT, the implantation rate was found to be significantly higher when the embryos were deposited 2 cm below the uterine fundus compared to when deposited 1 cm below the fundus (22,23).

Embryo after loading

Given the importance of ET technique during assisted reproductive technology cycles, some authors evaluated the effect of embryo afterloading subsequent to placement of the ET catheter on pregnancy rates vs. a standard direct ET. There was a

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trend toward an increase in pregnancy rate when an embryo afterloading technique was used. However a prospective randomized trial is needed to examine this issue (24).

Type of catheter

The benefit of one catheter over another is controversial. Some studies have reported better results with soft catheters. The type of catheter used can affect the pregnancy rate (PR). There is no significant difference in the PRs achieved by modern, soft, double-lumen ET catheters (26).

Ultrasound guided embryo transfer

In the past all uterine transfers were carried out as blind procedures. The use of ultrasound in such cases has proven beyond doubt that in a large number of cases the embryos are transferred either in the cervical canal or lower uterine cavity due to coiling or bending of catheter there.

After initiating a policy of ultrasound-guided miduterine cavity embryo transfer (ET) the incidence of retained embryos in the transfer catheter declined significantly. Different studies provide more evidence on the benefits of performing ETs under ultrasound guidance (27,28).

The experimental module described allows for direct visualisation of the mechanical passage of a catheter through the cervix and final placement of the tip in relation to the intravaginal visible part of the catheter (25).

Sallam and Sadek recently conducted a meta-analysis of RCTs and found that, compared to the clinical touch method, abdominal ultrasound-guided transfer significantly increases the clinical pregnancy rate and the on-going pregnancy rate (29). However ultrasound-guided embryo transfer does require a certain degree of eye-hand coordination and like any other new technique has a learning curve (5).

Prevention of uterine contraction

The use of a tenaculum straighten the canal and facilitate transfer, but stimulate uterine contractions due to release of oxytocin. Holding the cervix with a tenaculum was found to stimulate uterine junctional zone contractions affecting implantation of the transferred embryos. Even alternation in the normal contraction pattern may cause expulsion of the embryos from uterine cavity. In addition, progesterone administration starting on the day of oocyte retrieval induces a decrease in uterine contraction frequency on the day of ET (30).

It has been observed that, after embryo transfer, the embryos can move as easily toward the cervical canal as toward the fallopian tube (31). Thus the first and most important is to avoid the initiation of uterine contractility.

In knutzen *et al* study using radio-opaque dye, mimicking embryo transfer, it was found that the dye remained primarily in the uterine cavity in only 85% 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 problems (32).

These problems can be avoided by the use of soft catheter, gentle manipulation and by avoiding touching the fundus.

Mechanical pressure on the portiovaginalis of the cervix

Applying gentle mechanical pressure on the portiovaginalis of the cervix significantly improved the clinical pregnancy rate (33).

Sexual intercourse

It was shown that, the clinical pregnancy rate was not affected by sexual intercourse and, contrary to expectations; the implantation rate was significantly increased for patients who had sexual intercourse around the time of embryo transfer. No significant difference was found between the intercourse and abstain groups in relation to the PR (23.6 Vs 21.2%), but the proportion of transferred embryos that were viable at 6-8 weeks was significantly higher in women exposed to semen compared to those who abstained (11 Vs 7.7%) (34). Therefore, it seems that exposure to semen around the time of ET increases the likelihood of successful early embryo implantation and development.

ET with and without a full bladder

Performing ET with a full bladder to straighten the utero-cervical angle has been claimed to improve pregnancy and implantation rates (35). This effect being achieved indirectly by performing embryo transfer under ultrasound guidance in some centers.

However, no RCTs have so far been published to evaluate performing ET with and without a full bladder. Therefore, Performing ET with or without a full bladder is in doubt but probably full bladder

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straight the utero-cervical angle and may be improve pregnancy and implantation rates (36,37).

Type of withdrawal of the catheter

There was no statistically significant difference in pregnancy rate when the catheter was withdrawn immediately after ET compared to when it was left for 30 seconds in the uterus before its withdrawal (38).

Administration of antibiotics following ET

The effect of routine administration of antibiotics following oocyte retrieval or ET has not been studied by RCTs and is still a matter of debate (39).

The use of a fibrin sealant

Adding a fibrin sealant (glue) to the culture medium containing the embryos during ET, for improvement of pregnancy rate is inconclusive (40).

Conclusion

ET is the final crucial step in IVF treatment. However, it has been demonstrated by multiple investigators that great care and patience should be observed when performing an ET, because even small differences in methods may affect pregnancy rates. Dummy ET before the actual transfer can help the outcome of ART. Placement of the embryos 2 cm below the uterine fundus by ultrasound-guided ET is strongly suggested. Proper evaluation of the uterine cavity to ensure the proper placement of the embryos is recommended. Cervical mucus can be a serious obstacle in proper embryo replacement. All efforts should be made to avoid difficult embryo transfers provoking bleeding or uterine contractions. Since the very early days of IVF, the value of soft embryo transfer catheters has been recognized. Finally, the physician has to be absolutely sure that the catheter has passed the internal cervical os and the embryos are delivered gently inside the uterine cavity.

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