A concise overview of factors impacting success of embryo transfer in Assisted Reproduction Technology.

Walid K. Idriss¹, Reem A Albalawi², Abdel A Faraj², Alaa Mourtaja¹, Asma A AlBadran², Amal Y Zaman³, Majd E Alattasi¹, Mohammad M Kayali², Ayman M N Hassan², Jaffar Ali²,⁴

¹ Dr Khalid Idriss Hospital, Jeddah. Kingdom of Saudi Arabia
² ACU/IVF Department, Maternity and Children’s Hospital, MOH, Dammam, Eastern Province, Kingdom of Saudi Arabia
³ Taibah University, Al Madinah. Kingdom of Saudi Arabia
⁴ Department of Biomedical Science, Kulliyyah of Allied Health Sciences, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200, Kuantan, Pahang, Malaysia.

Abstract
The objective of this overview is to briefly summarize the large volume of knowledge that has been accrued on factors impacting embryo transfer (ET). The information summarized herein is anticipated to enable operators of ET acquaint and prepare themselves to perform ET in a scientific manner that could elicit the best possible outcome for the patient. The delivery of embryo(s) into the uterine cavity or embryo transfer (ET) is a critical step in assisted reproduction treatment. Every effort must be made to execute the ET procedure close to perfection leaving no room for procedural errors, less than optimal execution or negligence. Otherwise the whole effort will go to waste, resulting in enormous disappointment for both the patient and members of the treating team. It is a well-recognized the treatment outcome could be improved if ET was performed in a more skillful manner by operators that are also fully aware and knowledgeable of the factors that impact success of ET. About 16 factors that impact ET have been investigated by a number of workers. Fourteen factors have an impact while 2 factors (bloody catheter and mock ET) appear to be of no consequence. Removal of cervical mucus and hydrosalpinges; the use of soft catheters, ultrasound, seminal plasma, low molecular weight heparin and uterine relaxants; induction of mild endometrial injury, faster ET and expelling embryos 5 to 15mm from fundus, and embryo adherent compound(s) appear to impact outcome positively. The use of tenaculum, touching the fundus, or long bed rest after ET appears to impact outcome negatively.

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Correspondence: Walid Idriss, Dr Khalid Idriss Hospital, Jeddah. Kingdom of Saudi Arabia. E-mail: widriss@gmail.com
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Introduction

In assisted reproduction technology (ART) treatment the delivery of embryo into the uterine cavity, or commonly called “embryo transfer” (ET), is a critical step. Indeed the ET procedure is considered the most important step in the modality of IVF/ICSI. If ET is performed in a manner that is not close to perfection the patient may not get pregnant, as a consequence the whole effort will go to waste even if the embryos were of high quality and viable, not to mention the enormous disappointment for both the patient(s) and to the members of team involved in the generation of the embryos.

It therefore requires no emphasis that every effort must be taken to execute the ET procedure in a manner that is as close to perfect as possible leaving no room for procedural errors, less than optimal execution or negligence. In general, a successful ET is related to ease of transfer positioning inside the
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endometrial cavity with minimal manipulation (Schoolcraft et al., 2001). According to data presented at ESHRE 2009, only 32% of fresh ETs resulted in clinical pregnancies (Zygula et al., 2016) which raised the question whether this number could have been higher if ET was performed in a more skillful manner by operators that are also fully aware and knowledgeable of the factors that impact the success of ET. Implantation failure is one of the major factors limiting success of ART treatment.

The objective of this overview is to briefly but comprehensively address knowledge that has been accrued on factors impacting ET. This overview is intended to assist operators perform ET in a manner that could result in better outcomes for patients.

Impact of choice of ET catheter

The type of the ET catheter may play an important role in the outcome of IVF treatment. The ET catheter has been categorized as either soft or firm (or hard). If the ET catheter had a soft inner catheter it was classified as soft irrespective, while the others were classified as hard catheters. It is felt the ideal ET catheter be soft to avoid trauma to the cervix and endometrium. It is probable trauma caused by firm catheters and its consequent bleeding and/or induction of uterine contractions may be detrimental to the procedure. Meta-analyses of a number of workers (Gambadauro and Navaratnarajah, 2015; Brown et al., 2010) have pointed out that the choice of catheter could be one of the most important variables associated with outcomes of ET. A recent study also came to the same conclusion (Ren et al., 2016). Ren et al. (2016) compared the pregnancy outcomes when ET was performed with Cook Sydney IVF ET catheters (Cat.K-JETS-7019-SIVF) group 1 (n=949) and Frydman-CCD catheters (cat.131230301), group 2 (n=1115). These workers noted a significantly higher implantation rate, (34.40% vs. 26.92%, P<0.05), clinical pregnancy rate (CPR), (51.21% vs. 41.52%, P<0.05), live birth rate (42.57% vs. 33.09%, P<0.05) in group 1 compared to group 2. However, the abortion rate was not significantly different (11.93% vs. 15.98%, P>0.05).

Another study by Ruhlman and co-workers (Ruhlmann et al., 2015) compared ET using semi-rigid (group 1; n=363) and, soft and flexible (group 2, n=340) catheters from the same manufacturers (Frydman). They observed that ET was easier with the softer compared to the semi-rigid catheter. The difficult transfers were higher in group 1 than group 2 (5.27% vs. 3.41%, P<0.05). There was no difference in CPR and live birth rate.

The studies cited herein have noted catheters do impact outcome. Operators must be mindful of the differences and make a choice of catheter to ensure optimal outcome for their patients. The ASRM Practice Guidelines of 2017 on ET reported there is evidence to recommend the use of a soft ET catheter to improve IVF-ET pregnancy rates (ASRM, 2017)

Impact: Yes/softer catheters may be beneficial.

Impact of blood in/on ET catheter

It is well recognized that difficult ET could be traumatic and is associated with presence of blood in/on transfer catheter. After controlling of confounding variables, the presence of blood in the transfer catheter was found not associated with the likelihood of pregnancy. This indicates that the difficulty of the transfer was a strong negative predictor of pregnancy but not blood in the catheter (Plowden et al., 2016). Ruhlman et al. (2015) noted a significant negative impact of blood (9.9% vs. 3.8%) in catheter but unlike Plowden et al. (2016), no attempt was made to control confounding variables.

Given the mixed results of studies, there is insufficient evidence to state conclusively that the presence of blood on the catheter, once it’s drawn, is associated with implantation or pregnancy rate.

Impact: No/not harmful but caution prescribed

Impact of removing cervical mucus

Presence of excessive cervical mucus has long been recognized to impair ET by obstructing the tip of ET catheter, pulling back the embryos during catheter withdrawal, or expose the uterus to bacterial contamination (Otherwise provide evidence.refs for this statement). One RCT (Moini A et al., 2011) and one cohort study (Eskandar MA et al., 2007) showed improvement of pregnancy outcomes after removal of cervical mucus. However excessive cervical manipulation aiming to
remove all cervical mucus would probably stimulate uterine contraction with its concomitant adverse effect on pregnancy outcome. Seven cohort studies showed that the presence of mucus in or on the catheter (once it is withdrawn) does not adversely affect clinical pregnancy or live-birth rates (Nabi et al., 1997, Awonuga et al., 1998; Ebner et al., 2001; Alvero et al., 2003; Vissachers et al., 2007; Moragianni et al., 2010; Tiras et al., 2012, Listijono et al., 2013).

**Impact:** Yes/beneficial; caution prescribed

**Impact of the distance the catheter was introduced into the uterus, air bibles and touching uterine fundus on ET outcome**

Considerable attention has been given to the proper placement of the ET catheter inside the uterine cavity during the ET procedure. It is obvious that the introduction of the catheter into the uterine cavity need be performed with considerable care. Touching the fundus with the tip of the ET catheter may cause contractions and embryo expulsion (Tiras and Cenksoy., 2014). An overall higher ongoing pregnancy rate was observed in patients (n=1184 patients) whose embryos were released between 5 and 15 mm from the fundal endometrial surface compared to above 15 mm (Rovei et al., 2013). Another study also noted pregnancy rate is significantly influenced by transfer distance from the fundus where the pregnancy rate decreases from 46.2% in group B (distance: 15±2.5 mm) to 28.8% in group A (distance: 10±2.5 mm; p < 0.05; Ivanoski et al., 2012). A number of other findings support this recommendation. One is that of Lambers et al.,(2007) that observed that the position of the air bubbles after embryo transfer is related to pregnancy rate. They noted the highest pregnancy rates are found when the air bubbles end up closer to the fundus. The injection of 0.2ml of air immediately after ET appears to improve pregnancy rate (Madani et al., 2010). Another report using uterine model also noted the importance of distance (Eytan et al., 2007). They also noted the high speed of injection of embryos into uterine cavity could lead to ectopic pregnancy. They recommended adjustment of ET protocol to individual patient anatomy for optimal outcome. Groeneveld et al., (2012) also noted the importance of embryo placement in the uterus and speed of transfer.

**Impact:** Yes/distance important, positive impact if embryos expelled at around 5 to 15mm from fundus/touching fundus has negative impact

**Impact of tenaculum on ET outcome**

Manipulation with a tenaculum in the cervical area stimulates junctional zone contractions and is best avoided at time of ET (Lesny et al., 1999). Use of tenaculum is associated with expulsion of the transferred embryo (Tiras and Cenksoy., 2014).

**Impact:** Yes/negative/harmful

**Impact of removal of hydrosalpinges**

Hydrosalpinges are found in 10–30% of couples presenting with infertility from tubal factors (Andersen et al., 1994; Blazar et al., 1997; Katz et al., 1996; Murray 1998; Strandell et al., 1994). In addition to its role in infertility, hydrosalpinges have an adverse effect on the success of in vitro fertilisation (IVF). Live birth rates of infertile women with hydrosalpinges undergoing IVF are reduced by 50% in comparison to controls, i.e. infertile women without hydrosalpinges (Camus et al., 1999; Zeyneloglu et al., 1998).

Management of hydrosalpinges, usually by laparoscopic procedures (salpingectomy/proximal tubal occlusion), before IVF leads to improved outcomes (Johnson et al., 2010; ASRM. Practice Committee of American Society for Reproductive Medicine in collaboration with Society of Reproductive Surgeons. A systematic review and Cochrane review performed by Johnson et al.(2002) demonstrated that the odds of pregnancy [odds ratio (OR) = 1.75, 95% confidence interval (CI) 1.07-2.86] and of ongoing pregnancy and live birth (OR = 2.13, 95% CI 1.24-3.65) were increased with laparoscopic salpingectomy for hydrosalpinges prior to IVF.

**Impact:** Yes/beneficial

**Impact of difficult ETs on outcome**

Although ET is generally an easy procedure, difficulties can occur, resulting in more time spent on the procedure which invariably may
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require additional manoeuvres, change of catheter to a more rigid type or the use of tenaculum or other instruments. Difficult transfers are associated with a fall in pregnancy rates (Larue et al., 2017a,b). A retrospective observational study was performed involving a total of 7,714 ETs. The CPR was significantly higher in the cases of easy ET compared with difficult ET [38.2% vs. 27.1%] (Kava-Braveman et al., 2017). The CPR decreases progressively with the use of additional manoeuvres during ET.

About 5 to 7% of the ET’s have been reported to be extremely difficult to perform (Wood et al., 1995; Tur-Kaspa et al., 1998; Fereri et al., 2018). In rare instances extremely difficult or impossible transcervical ETs have been abandoned and Towako transmyometrial ET was performed instead under transvaginal US guidance (Towako et al., Ferreri et al., 2018). It is of interest to note the pregnancy rate was similar to the easier transcervical transfers following transmyometrial ET (Biervaliet et al., 2002).

Impact: Yes/negative when difficult

Impact of ultrasound ETs on outcome

Transabdominal ultrasound guided ET is in part intended to reduce the likelihood of trauma that could arise due to penetration by the catheter. Use of ultrasound has the advantage of obviating a blind approach to ET and in all likelihood may prevent direct contact of the catheter with the endometrium or fundus. A systematic Cochrane review investigated 21 studies on the effect of ultrasound on ET. The evidence from this investigation indicates that ultrasound guidance improves the chance of (i) clinical pregnancies (OR 1.31, RR 1.7, CI 1.45; 20 trials ; N=6711) and (ii) live birth/ongoing (OR 1.47, RR 1.3, CI 1.65 ; 13 trials; N=5859 women) compared with clinical touch, without increasing the chance of multiple pregnancy, ectopic pregnancy, or miscarriage (Brown et al., 2016). The retrospective observational study involving a total of 7,714 ETs undertaken by Kava-Braveman and co-workers have also shown poor ultrasound visualization contributed to significantly decreased clinical pregnancy rates (Kava-Braveman et al., 2017). The ASRM Practice Guidelines on ET (ASRM, 2017) based on 10 RCTs recommended transabdominal ultrasound guidance during ET. It has shown to improve clinical pregnancy and live-birth rates. Ultrasound energy may have harmful bioeffect on embryo but this remains to be conclusively elucidated (Abramovicz, 2017)

Impact: Yes/beneficial

Impact of endometrial injury

A Cochrane review found that endometrial injury performed prior to the ET cycle improves clinical pregnancy and live birth rates in women undergoing ART. However it appears imprudent to perform endometrial injury on the day of oocyte retrieval because it led to significantly reduce clinical and ongoing pregnancy rates. There is insufficient evidence regarding the effect of endometrial injury on multiple pregnancy or miscarriage and none on adverse events such as pain and bleeding (Nastri et al., 2012).

The systematic review of Zygula et al. (2016) noted that a majority of trials showed positive impact of endometrial injury on IVF outcome, but there is still a lack of strong evidence to support routine local endometrial injury in women prior to IVF treatment.

Impact: yes/beneficial but caution prescribed

Impact of mock trial (MET)

The study of Katariya et al., (2007) demonstrated that the timing of mock ET (MET) does not affect IVF implantation or pregnancy rates. It is also suggested performing a MET at the time of oocyte retrieval, 3 to 5 days before ET does not have a deleterious effect on the endometrium. Of interest is the finding that there was a statistically significant difference in the uterine cavity length at the time of early MET when compared with MET performed at oocyte retrieval. This suggests that the uterus is dynamic, such that measurements of the uterine cavity may indicate a longer length when measured closer to the time of actual ET. It is possible that assessment of the uterine cavity length closer to the time of the actual ET may be more accurate and identify yet another potential variable associated with ET success. Furthermore Miller et al. (2007) concluded that the uterine depth significantly differed
between the blind pre-cycle mock transfer measurement and the ultrasound-guided ET measurement. The mock transfer may predict a difficult ET but it is an inaccurate predictor of the final ET depth.

**Impact: Nil/no impact**

**Impact of seminal plasma**

A meta-analysis involving 2,128 women undergoing IVF investigated the effect of seminal plasma on ET outcome. Women who had exposure to seminal plasma either through sexual contact or injection of seminal plasma around time of oocyte pick-up were the test group. The investigators noted that sexual intercourse led to significantly higher rate of CPR compared to controls (30.0% vs 25.1%; RR 1.20, 95% CI 1.04 to 1.39). The study also observed that seminal plasma when injected just after oocyte pick-up, also showed significantly higher CPR (46.3% vs 37.2%; RR 1.23, 95% CI 1.05 to 1.45). This led the investigators conclude that intravaginal or intracervical application of seminal plasma around the time of oocyte pickup was associated with higher CPR (Saccone et al., 2017).

**Impact: Yes, beneficial**

**Impact of intrauterine injection of low-molecular-weight heparin**

Heparin can modulate proteins, and influence the processes involved in implantation and trophoblastic development. This study aimed to assess the improvement of clinical pregnancy and implantation rates after local intrauterine injection of low-molecular-weight heparin (LMWH) in patients undergoing intracytoplasmic sperm injection (ICSI).

A Cochrane review of three randomized clinical trials (RCTs) was undertaken to determine the impact of intrauterine injection of low molecular heparin on ET outcome. The review noted statistically significant differences in the clinical pregnancy and live birth rates between both groups (p=0.182 and p=0.096, respectively). The Cochrane reviewers concluded that the intrauterine administration of heparin in assisted reproduction treatment (ART) cycles may improve the live birth rate in women undergoing assisted reproduction (Akther et al., 2013). It is noteworthy that another study performed three years later found no such effect (Kamel et al., 2016).

**Impact: yes/beneficial**

**Impact of time taken for performing ET and age on outcome**

The study of Cetin et al. (2010) attempted to determine the effect of time taken to perform ET on treatment outcome. Their findings were statistically significant (P = 0.020) different for the different time periods required to perform ET. The clinical pregnancy rates for three different time intervals taken to perform ET were as follows:

- 31.6% for the <44 seconds interval - (Fast ET)
- 25.9% for the 45–59 seconds interval - (Moderately fast ET)
- 23.6% for the 60 seconds interval - (Slow ET)

According to logistic regression analyses, the odds of failed pregnancy increased by 1.61 times for ET durations longer than 60 seconds and odds ratios of a failed pregnancy was 1.53 (95% CI 1.18–1.99) in the 35 year age group, 1.49 (95% CI 1.05–2.12) for patients with fewer than five oocytes and 3.38 (95% CI 2.10–5.43) for those with fewer than two transferred embryos. In conclusion the investigators suggest that to increase the likelihood of a successful pregnancy in women over 35 years of age, the duration of ET must be kept below 60 seconds (Cetin et al., 2010).

**Impact: yes/ less time taken associated with better outcome**

**Impact of uterine relaxants**

One of the factors affecting success rates in assisted reproductive techniques is the side effects of ET such as cramps and contractions. The usefulness of muscle relaxants such as Hyoscine or Indomethacin (rectal suppository) or Piroxicam (oral) administration 30 minutes prior to ET has been investigated. In conclusion, Hyoscine but not Indomethacin or Piroxicam administration 30 minutes prior to ET increased
pregnancy rates. Piroxicam can prevent or reduce uterine cramps after the procedure (Sohrabvand et al., 2014) however it is suggested caution be exercised in the use of muscle relaxants in ET until a larger study is completed.

**Impact:** positive / but caution prescribed

**Impact of bed rest following ET**

A systematic review and meta-analysis was performed to determine the effect of bed rest following ET (Craciunas and Tsampras, 2016). The four RCTs included in the investigation that involved 757 women. The findings suggest that bed rest following ET will not improve clinical pregnancy and live birth rates, but on the contrary reduced the implantation rate. The findings of this systematic review and meta-analysis are in agreement with previous reports (Kucuk et al., 2013; Li et al., 2011) that suggest that bed rest is not beneficial following ET and may in fact be harmful as it might negatively affect the outcome of IVF/ICSI cycles through stress and anxiety mechanisms.

**Impact:** Negative/harmful

**Adherence compounds in ET Medium**

The Cochrane review on the use of embryo adherence compounds are varied and is considered conflicting (Bontekoe et al., 2014).

Following the report of Bontekoe et al (2014) a subsequent RCT by Fancsovits et al. (2015) that investigated 581 cycles did not demonstrate beneficial effects of the adherence compounds on implantation, pregnancy or live birth rates, instead investigated noted a higher birthweight in these patients. Although some of the published work showed a propensity towards beneficial effect of adherence compounds however most commentators and workers (Bontekoe et al., 2014; Datta et al., 2015; Harper et al., 2017) are of the opinion well-designed more robust studies are needed to evaluate the usefulness of adherence compounds in ET medium with respect to eSET for the possibility of reducing the multiple pregnancy rate.

**Impact:** yes, positive; but usefulness remains to be proven conclusively.

**Discussion**

As described herein numerous factors impact ET. A summary of the impact of various factors affecting ET is given in Table 1. Lack of knowledge pertaining to factors that affect ET or a dearth of careful attention to details or a combination of both can result in failure of the treatment procedure. It is not uncommon to see ET being performed in a hurried manner by impatient operators who have tight schedules to meet or by nature are impatient or is performed by trainees who lack both the knowledge and preparation to perform such procedures. Such transfers invariably lead to lower pregnancy rates than those performed in a meticulous manner by workers that are knowledgeable of the factors that affect success of ET. It is therefore crucial operators are aware of the factors that could impact ET both positively and negatively so that they will be well-prepared to perform the procedure in a manner optimal for embryo implantation.

Operators must be mindful of the enormous disappointment to the couple if the treatment cycle failed as a direct consequence of poorly executed ET; especially in patients with limited financial resources. Also operators must bear in mind the colossal meticulous effort and assiduous diligence that has been invested by the entire team to generate the embryos for ET.
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Therefore final step in the ART treatment procedure, which is the ET, must do justice to both the patients and the team that has worked hard to generate the embryos. ART is an emotionally-charged treatment modality. In empathy towards the patients that may be under considerable stress during the treatment procedure the entire team and in the present context, the ET operators, must be aware of the factors impacting ET and execute their individual responsibilities with meticulous diligence and compassionate care to ensure every conceivable factor that could lead to failure is assiduously avoided so that the patients get the best possible chance of becoming pregnant.

Trainees need to acquire knowledge on factors that impact the success or failure of ET alongside simultaneous exposure to the ET procedure to become completely familiar with the procedure, initially as an observer and subsequently assist in the performance of at least 50 ETs before they attempt the procedure under supervision.

A number of other factors that may impact ET remains to be investigated.

Conclusion

There are a number of factors that impact ET outcome in ART treatment. It would be of considerable interest and prudent for operators of ET to acquire knowledge on the factors that could impact ET positively or negatively so that they may be able to perform ET that could elicit the best possible outcome for the patient.

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Table 1: Summary of factors that impact outcome of embryo transfer

<table>
<thead>
<tr>
<th>Sr no.</th>
<th>Factor Description</th>
<th>Impact</th>
<th>Concluding Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Choice of ET catheter</td>
<td>Yes</td>
<td>Softer catheters may be beneficial</td>
</tr>
<tr>
<td>2</td>
<td>Blood in/on ET catheter</td>
<td>No</td>
<td>Not harmful but CP</td>
</tr>
<tr>
<td>3</td>
<td>Removing cervical mucus</td>
<td>Yes</td>
<td>Positive; Beneficial; CP</td>
</tr>
<tr>
<td>4</td>
<td>Distance from or touching uterine fundus</td>
<td>Yes</td>
<td>Distance crucial</td>
</tr>
<tr>
<td>5</td>
<td>Use of tenaculum</td>
<td>Yes</td>
<td>Negative &amp; Harmful</td>
</tr>
<tr>
<td>6</td>
<td>Removal of hydrosalpinges</td>
<td>Yes</td>
<td>Beneficial</td>
</tr>
<tr>
<td>7</td>
<td>Difficult embryo transfer</td>
<td>Yes</td>
<td>Negative if difficult</td>
</tr>
<tr>
<td>8</td>
<td>Use of ultrasound</td>
<td>Yes</td>
<td>Positive, Beneficial</td>
</tr>
<tr>
<td>9</td>
<td>Induced minor endometrial injury</td>
<td>Yes</td>
<td>Positive, Beneficial/ but CP</td>
</tr>
<tr>
<td>10</td>
<td>Mock embryo transfer (MET)</td>
<td>No</td>
<td>No effect</td>
</tr>
<tr>
<td>11</td>
<td>Seminal plasma on ET</td>
<td>Yes</td>
<td>Positive, beneficial</td>
</tr>
<tr>
<td>12</td>
<td>IUI injection of heparin</td>
<td>Yes</td>
<td>Positive, beneficial</td>
</tr>
<tr>
<td>13</td>
<td>Time taken to perform ET</td>
<td>Yes</td>
<td>Less time led to better outcome</td>
</tr>
<tr>
<td>14</td>
<td>Uterine relaxants at ET</td>
<td>Yes</td>
<td>Positive but CP</td>
</tr>
<tr>
<td>15</td>
<td>Bed rest following ET</td>
<td>Yes</td>
<td>Negative, harmful</td>
</tr>
<tr>
<td>16</td>
<td>Adherence compound in ET medium</td>
<td>Yes</td>
<td>Positive, need more studies</td>
</tr>
</tbody>
</table>

CP= Caution prescribed


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