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Embryo transfer techniques

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A B S T R A C T

Embryo transfer is the final and rate-limiting step of the assisted reproductive technique. Few advances have occurred in the last few decades with regard to this procedure. Studies conducted thus far have focused on factors and interventions taking place before, during and after this procedure. These factors are highly varied and range from methods to improve the psychological state of the patients to methods aimed at reducing uterine contractility and methods aimed at optimising the precise transfer of the embryo. The key question is which factors and interventions have thus far been proven to increase pregnancy rates and live birth rates. This paper aims to review the evidence relating to embryo transfer techniques in a systematic manner with a view to provide practical recommendations to practitioners involved in the procedure.

Many practitioners believe that embryo transfer is one of the most important procedures in *in vitro* fertilisation (IVF) treatment. In the manuscript, we have attempted to review evidence relating to the embryo transfer technique under the following headings:

1. Prior to embryo transfer

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2. During embryo transfer
3. Post embryo transfer

Prior to embryo transfer

Does measuring stress prior to embryo transfer predict reproductive outcomes?

The effect of stress on the reproductive outcomes of women with infertility remains a point of debate. We have recently attempted to correlate both biological stress and psychological stress to reproductive outcomes at the time surrounding embryo transfer [1]. The former was measured using salivary α-amylase and the latter using dedicated psychological questionnaires. Interestingly, in 197 women examined, we found that both biological and psychological stress parameters did not correlate with pregnancy rates or miscarriage rates [1]. It appears therefore that measuring stress scores prior to embryo transfer does not predict reproductive outcomes and that stress reduction strategy is not necessary prior to embryo transfer.

Does acupuncture prior to embryo transfer improve reproductive outcomes?

The use of acupuncture has gained increased popularity as an adjuvant therapy used in conjunction with ART and particularly embryo transfer. The mechanisms of action remain largely unknown, and the literature has been generally divided according to its usefulness and evidence for use within this context.

Andersen et al. (2010) performed a prospective randomised controlled trial (RCT) including a total of 635 patients undergoing ART, of which 314 underwent acupuncture 30 min prior to and 30 min post embryo transfer, whereas 321 underwent placebo acupuncture in a similar timeframe. In the acupuncture group and the placebo group, the ongoing pregnancy rates were 27% (95% confidence interval [CI] 22–32) and 32% (95% CI 27–37), whereas the live birth rates were 25% (95% CI 20–30) and 30% (95% CI 25–30), respectively, with no statistically significance demonstrated [2]. Interestingly, So et al. (2009), using a similar RCT design including 370 patients, found the pregnancy rate to be significantly lower in the acupuncture group than in the placebo acupuncture group (43.8% versus 55.1%; $P < 0.05$) [3]. Indeed, the available meta-analyses including all relevant studies on the subject found no evidence that acupuncture improves live birth or pregnancy rates in assisted conception [4]. In fact, the most recent meta-analysis available in the literature suggested that acupuncture performed on the day of ET may be associated with a reduced risk of clinical pregnancy (0.87, 95% CI 0.77 to 0.98) [5]. Therefore, acupuncture prior to and post embryo transfer appears not to be associated with improved reproductive outcomes.

Does measuring uterine contractility prior to embryo transfer predict reproductive outcomes?

The importance of uterine contractility has long been implicated in the process of natural conception. More than two decades ago, Ijland et al. (1997) reported that uterine contractility in conception cycles was less than that of non-conception cycles [6]. The following year, Fanchin et al. (1998) observed that uterine contraction frequency prior to embryo transfer was inversely related to implantation rate and clinical pregnancy rate [7]. A few years later, Zhu et al. (2012) reported that the uterine contractility and pattern differed significantly between natural and stimulated ART cycles [8]. Similar to Fanchin et al., they also went to confirm in their subsequent study that uterine contractility was inversely correlated to pregnancy rates in both fresh and frozen embryo transfer cycles [9].

In our own prospective cohort study of 286 women undergoing fresh embryo transfer after IVF, we measured uterine contraction frequency immediately before (−5 min), immediately after (+5 min) and 60 min after embryo transfer. We found that mean ± SD uterine contraction frequency at −5 min was 1.8 ± 1.1 contractions per min, increasing significantly to 2.0 ± 1.1 at +5 min ($P < 0.05$) and returning

back to baseline 1.8 ± 1.1 at +60 min. Although live birth rates were reduced for women with high versus low uterine contractility 5 min prior to embryo transfer (26.5% versus 38.8%, respectively; $P < 0.05$), logistic regression analysis suggested that uterine contractility immediately after embryo transfer was the strongest predictor of reproductive outcome ($P < 0.01$) and may help to identify women who could potentially benefit from the use of muscle relaxant therapy to improve outcome [10]. These results all point to the fact that uterine contractility around the time of embryo transfer is significantly correlated to reproductive outcomes.

Should we use oxytocin antagonists (Atosiban) prior to embryo transfer?

Given the evidence and physiological plausibility of increased uterine contractility adversely affecting the reproductive outcomes, the use of anti-contractile agents has been investigated as a potential treatment strategy to improve reproductive outcomes. Although there has been a debate regarding the presence and role of oxytocin receptors in the non-gravid uterus, Kunz et al. (1998) have shown a significant increase in uterine contractility following oxytocin injections in the early and mid-follicular phases [11]. In addition, Wildt et al. (1998) have reported that oxytocin administration may change the direction of the uterine contractions, which undoubtedly may also play a role in the implantation process [12]. Therefore, it is not a surprise that the use of the oxytocin antagonist Atosiban around the time of embryo transfer has been the subject of a number of clinical studies.

Moraloglu et al. (2010) were one of the first groups to compare Atosiban versus placebo within the context of an RCT including 180 women. They started the infusion 30 min prior to embryo transfer and continued it up to 2 h post transfer. They found the implantation rate and clinical pregnancy rate to be 20.4% and 46.7% in the Atosiban group and 12.6% and 28.9% in the placebo group, respectively, being significantly reduced in the placebo group ($P = 0.01$) [13]. However, Ng et al. (2014) in a multicentre trial of 800 unselected women using a similar protocol found no significant difference in the live birth rate between the Atosiban and placebo groups (39.8 versus 38.0%, $P = 0.612$). No significant differences were also found between the two groups in the positive pregnancy test, clinical pregnancy, ongoing pregnancy, miscarriage, multiple pregnancy, ectopic pregnancy rates and implantation rate per woman [14].

In a prospective observational study including a selected group of 71 women with recurrent implantation failure (i.e. no previous implantation or clinical pregnancy from an average of 4.8 previous embryo transfers with a mean of 12 top-quality embryos), Lan et al. (2012) used the Atosiban protocol as mentioned above. They reported a rather favourable implantation rate of 13.9% and clinical pregnancy rate of 43.7%, suggesting that Atosiban may have a role to play in selected women with recurrent implantation failure [15].

The results of an early meta-analysis in 2016 suggested that the administration of Atosiban on the day of embryo transfer may improve the implantation rate but not the clinical pregnancy rate or miscarriage rate. Understandably, additional, large-scale, prospective, randomised studies were recommended [16]. Another recent meta-analysis the following year found that Atosiban was associated with higher implantation (OR = 1.63, 95% CI: 1.17–2.27; $P = 0.004$) and clinical pregnancy (OR = 1.84, 95% CI: 1.31–2.57; $P < 0.001$) rates but not live birth rate. When a further subgroup analysis was performed in women with recurrent implantation failure, the Atosiban group had significantly higher implantation (OR = 1.93, 95% CI: 1.45–2.57; $P < 0.001$), clinical pregnancy (OR = 2.48, 95% CI: 1.70–3.64; $P < 0.001$) and live birth (OR = 2.89, 95% CI: 1.78–4.67; $P < 0.001$) rates than the other group. The authors concluded that Atosiban may be more appropriate for women with recurrent implantation failure, but it plays only a limited role in improving pregnancy outcomes in the unselected general population of women undergoing ART [17]. Overall, although promising, further large RCTs with varying protocols are required in selected groups of women undergoing ART to elucidate the role of oxytocin antagonists such as Atosiban on women undergoing embryo transfer.

Should we use other agents that may reduce uterine contractility?

Other than oxytocin antagonists, there are several other agents that may affect uterine contractility through various pathways. These agents include progesterone, anti-cholinergic agents (e.g. hyoscine butylbromide), beta-adrenergic receptor antagonists (e.g. terbutaline and ritodrine), prostaglandin

synthetase inhibitors (e.g. indomethacin, piroxicam and aspirin) and nitric oxide donors (e.g. nitroglycerine and glyceryl trinitrate). A recent comprehensive review and meta-analysis by Kuijsters et al. (2017) summarised all the available evidence in the literature regarding these agents [18].

The first worth mentioning is progesterone, the use of which is also implicated in luteal phase support, as luteal phase insufficiency is traditionally thought to be due to inadequate production of progesterone after ovulation [19]. However, despite the widespread use of progesterone as an agent for luteal phase support, progesterone use prior to embryo transfer may also have an additional beneficial effect through reducing uterine contractility. A well-conducted RCT has examined this issue by assessing the uterine contractility in a group of women receiving progesterone on the day of oocyte retrieval versus on the evening of the embryo transfer (n = 43 and n = 41, respectively). It was found that whereas uterine contraction frequency was similar in both groups on the day of hCG trigger (4.6 ± 0.3 and 4.5 ± 0.3 contractions per minute, respectively), women receiving progesterone on the day of oocyte retrieval versus on the evening of embryo transfer showed decreased uterine contraction frequency on the day of embryo transfer (2.8 ± 0.2 versus 4.2 ± 0.3 contractions per minute) [20]. Similarly, when looking at the meta-analysis of 7 RCTs (total n = 841) using progesterone versus placebo prior to embryo transfer, there was a significant increase in the pregnancy rate relative risk (RR) 1.68 (95% CI 1.23–2.28) in the progesterone group [18].

In the same meta-analysis, when evaluating the use of anticholinergic agents, 2 RCTs (total n = 186) showed a significant increase in the pregnancy rate RR 1.82 (95% CI 1.10–3.03); for the use of beta-adrenergic receptor antagonists, 3 RCTs (total n = 364), showing no significant increase in the pregnancy rate RR 1.14 (95% CI 0.80–1.63); for the use of prostaglandin synthetase inhibitors, 7 RCTs (total n = 1152) showed a significant increase in the pregnancy rate RR 1.35 (1.15–1.58) and finally, for the use of nitric oxide donors, 3 RCTs (total n = 358) showed no significant increase in the pregnancy rate RR 1.03 (95% CI 0.76–1.39). However, it is worth mentioning that for all agents evaluated in this meta-analysis, except progesterone, the quality of evidence was considered to be low or very low [18]. Therefore, the results should be interpreted with caution. Another interesting agent that would be worth evaluating is the calcium channel blocker Nifedipine. There is thankfully a double-blind RCT currently underway assessing the use of nifedipine 30 min prior to and 30 min post embryo transfer versus placebo, the result of which is awaited with interest [21].

Overall, although promising, further well-conducted RCTs are required to elucidate the value of using agents with a muscle relaxant property in different populations of women undergoing embryo transfer.

Should we use prophylactic antibiotics prior to embryo transfer?

Genital infections, endometritis and altered microbiome have been implicated in infertility [22]. Therefore, the simple question of whether to use prophylactic antibiotics prior to embryo transfer seems to be a pertinent one. The current evidence from one RCT suggests that although prophylactic antibiotic use prior to embryo transfer may significantly reduce catheter contamination rates versus no antibiotic use (49.4% versus 62.3%; $P < 0.05$), there is no difference in clinical pregnancy rates between the two groups (36.0 versus 35.5%). Interestingly, the authors did find a significant association between the level of bacterial contamination and clinical pregnancy rates ($P < 0.05$), although this may reflect other underlying physiological processes [23]. Although this RCT may suggest that there is no need for prophylactic antibiotics prior to embryo transfer, more detailed studies examining the screening of subclinical endometritis, patterns of microbiome and use of antibiotics are likely to be of significance in the future.

Should we perform a mock embryo transfer prior to embryo transfer?

Mock embryo transfer has been described for almost 30 years, the proposed benefit being that of predicting difficult embryo transfers and allowing the appropriate selection of embryo catheter [24]. The timing of the mock transfer (i.e. during oocyte retrieval, 3–5 days prior or immediately prior to actual embryo transfer) does not appear to have an adverse effect on reproductive outcomes [25,26]. More recent studies have suggested that the use of ultrasound guidance during a mock embryo transfer may be additionally beneficial by assessing the cavity depth more accurately [27]. However, the value of mock embryo transfer to our knowledge has not been tested within the context of an RCT; therefore,

it remains unknown whether its proposed benefits translate into improved reproductive outcomes. Alternative novel ultrasound imaging techniques such as 3D ultrasound may allow identification of difficult embryo transfers through performing a 'virtual embryo transfer', although future studies will have to assess the potential clinical benefits (Fig. 1).

During embryo transfer

Should we use prophylactic antibiotics prior to embryo transfer?

Although there are limited data on this subject, one RCT (total n = 530) showed an improvement in clinical pregnancy rate (39.2% versus 22.6%; P < 0.001) and live birth rate (33.6% versus 17.4%; P < 0.001) when removing cervical mucus versus not removing cervical mucus, respectively [28]. A prospective controlled study of 286 women also reached a similar conclusion, with the clinical pregnancy rate being significantly higher for removing versus not removing cervical mucus (OR 2.18, 95% CI 1.32–3.58; P < 0.001) [29]. Once again, future studies examining the microbiome of the female genital tract and the vagina and cervical canal in particular may shed more light into the mechanisms by which we may improve reproductive outcomes within this context.

Should we use soft or hard embryo transfer catheters?

Owing to the variety of different embryo transfer catheters available, this question is difficult to examine. One way to answer this question is by comparing soft versus hard transfer catheters. Two RCTs are available in the literature using this comparison. The trial of McDonald and Norman (total n = 650) showed that there was a significantly higher pregnancy rate in the group treated with a soft catheter than with a hard catheter (29.6 versus 20.5%; P < 0.01) [30]. Similarly, in the trial of van Weering et al. (total n = 2059), the ongoing pregnancy rate was significantly higher in the soft catheter group than in the hard catheter group (27.1 versus 20.5%; P < 0.01) [31]. A meta-analysis performed by the ASRM Practice Committee using these two studies found a significant increase in pregnancy rates with the use of soft catheters (RR 1.36, 95% CI 1.16–1.59), therefore recommending their use [32].

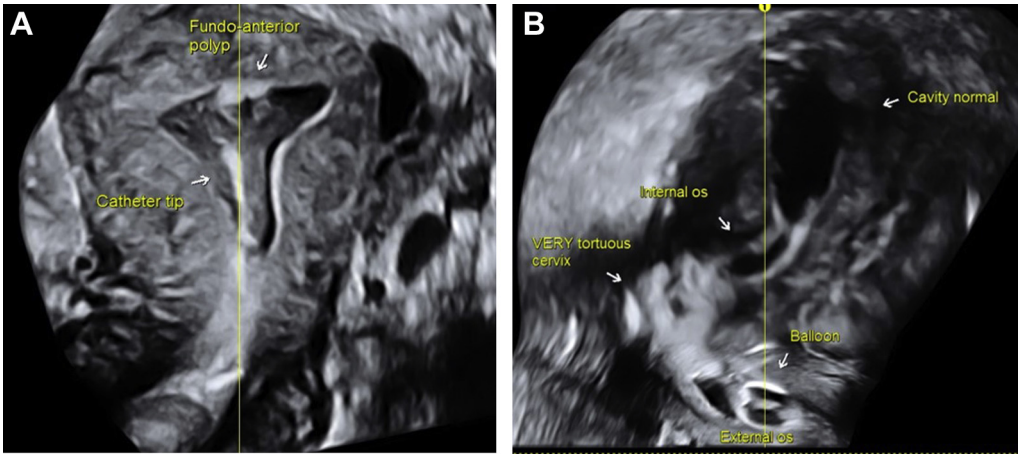

Fig. 1. 3D ultrasound 'virtual embryo transfer' performed during a saline infusion ultrasound assessment. 3D ultrasound OmniView function is used to map out the path of the cervical canal into the endometrial cavity. Image A shows a likely easy embryo transfer, whereas image B shows a likely difficult embryo transfer.

Should we use ultrasound guidance during embryo transfer?

Ultrasound guidance is nowadays used by most centres for embryo transfer owing to its widespread availability, reducing cost and, most importantly, evidence base. This is supported by numerous RCTs and two recent meta-analyses demonstrating improved reproductive outcomes [33,34].

The most recent meta-analysis by Brown et al. (2016) concluded that compared to no ultrasound use, ultrasound guidance was associated with a significant increase in the chance of a clinical pregnancy (OR 1.31, 95% CI 1.17–1.45) and an increased chance of a live birth/ongoing pregnancy (OR 1.47, 95% CI 1.30–1.65). They further estimated that for women with a chance of a live birth/ongoing pregnancy, with 23% of them using no ultrasound, this would increase to between 28% and 33% if ultrasound guidance was to be used. The quality of evidence was considered as low, but 20 trials (total n = 6711) were included in the final analysis [33].

When assessing whether transvaginal versus transabdominal ultrasound guidance conveys any benefit, three studies identified by the meta-analysis of Teixeira did not demonstrate significant improvements in reproductive outcomes [34]. When considering whether 3D ultrasound guidance may improve reproductive outcomes, preliminary reports appeared promising [35–37]; however, we performed a prospective RCT on a total of 481 women who were randomised to either a 3D or a 2D ultrasound-guided embryo transfer, the results of which did not follow suit. The outcome of our trial showed that there was no significant difference in the ongoing pregnancy rate between the 3D and 2D ultrasound embryo transfer US groups (35.4% versus 37.1%; P = 0.70). There were also no significant differences in terms of positive hCG rate, biochemical pregnancy rate, implantation rate, clinical pregnancy rate, miscarriage rate, ectopic pregnancy rate and multiple pregnancy rate [38]. Therefore, although transabdominal ultrasound guidance during embryo transfer does appear to improve reproductive outcomes, the use of transvaginal or 3D ultrasound does not appear to convey any additional benefit.

Should we inject the embryos at a particular location during transfer?

Since the introduction of ultrasound guided embryo transfer, studies have examined the relationship between the distance of the catheter tip to the fundus and the chance of pregnancy. Initially, most authors suggested that placing the embryo in the lower part of the uterine cavity may result in higher pregnancy rates [39–41], whereas others suggested that a central location is most favourable [42,43] and others report no differences [44]. A meta-analysis including 3 RCTs suggested a trend for improved pregnancy rates with catheter placements further away from the fundus (i.e. greater than 2 cm from the fundus), although it concluded that the published data remain limited [45]. The ASRM Practice Committee recently recommended placement of the catheter tip in the upper or middle (central) part of the uterine cavity, greater than 1 cm from the fundus, basing their conclusion from 3 RCTs and 4 cohort studies [32].

It is worth noting that following on from publications assessing the catheter tip position, more recent publications have focussed on visualising the position of the air bubbles that have often been loaded into the transfer catheter along with the embryo(s) [46]. These air bubbles are surrogate markers of the embryo location often apparent on ultrasound if the embryologist loads the drop of medium containing the embryo(s) along with an air bubble followed by another drop of medium [31]. It has been stipulated that the location and migration of these air bubbles may help to identify the exact position of the embryo following embryo transfer [47]. The air bubble studies seem to suggest that pregnancy rates are highest when the air bubbles are closer to the fundus [46–48], which is at odds with several of the catheter tip studies. However, it should be noted that the embryo position may change after transfer into the uterine cavity. It is possible that the frequency and direction of uterine contractions immediately after embryo transfer may move the embryo away from the position where it was transferred, especially bearing in mind that the process of implantation may occur several hours or even days later.

Indeed, in our prospective study employing the use of 3D US at the time of embryo transfer and subsequent early pregnancy, we found that, overall, only 40.8% (29/71) of embryos implanted at the

location where the air bubbles were visualised at 1 min after ET, and 50.7% (36/71) implanted where the air bubbles were visualised at 60 min after ET (Cohen's kappa coefficients 0.21 and 0.37, respectively; comparison of agreement values: $P = 0.28$) [49]. In addition, in another prospective study assessing the embryo flash position and migration as visualised with 3D ultrasound (US) within 60 min of embryo transfer, we found that the location of the embryo flash and the direction of its movement at 60 min, but not at 1 or 5 min after transfer, was associated with clinical pregnancy. Interestingly, there was no significant association between the embryo position or movement and the pregnancy rate at 1 and 5 min. At 60 min, however, the pregnancy and implantation rates among subjects with embryo flashes located <15 mm from the fundus were significantly higher than those with embryo flashes located >15 mm from the fundus (46.5 and 32.8% versus 25.8 and 18.2%, respectively; $P < 0.05$). The pregnancy and implantation rates when the embryo flash was seen moving towards the cervix (25.0% and 15.0%) were significantly lower ($P < 0.05$ and $P < 0.01$, respectively) than those remaining static (55.2% and 37.7%) or moving towards the fundus (45.5 and 32.8%) [50]. Overall, it appears that although transferring the embryo into the central or upper part of the uterine cavity seems intuitive and reasonable, embryo migration due to factors such as uterine contractility may mean that the initial location of transfer is not as important as we initially believed.

How should we avoid undue uterine contractions during embryo transfer?

Although it is known that increased uterine contractions are associated with adverse reproductive outcomes, the ideal pharmacological methods and transfer techniques that may reduce the contractility and improve the clinical outcomes remain unknown. The use of different agents to reduce contractility has been discussed in other sections of this chapter. In terms of mechanical stimulation, it has been shown that the insertion of an embryo transfer catheter itself increases uterine contractions [10]. Therefore, the embryo transfer procedure should be performed as gently as possible throughout, from the removal of the cervical mucous to the insertion and removal of the embryo transfer catheter. Equally, overdistention of the urinary bladder has been shown to be associated with increased uterine contractions and should therefore be avoided [10].

At what rate should we inject the embryos?

The injection speed during embryo transfer is understandably notoriously difficult to standardise and measure. In a study aimed at examining the variation in injection speeds in manual embryo transfer, seven laboratory technicians were asked to perform simulated transfers by the conventional embryo transfer technique. Their injection speeds were compared with those using a pump-regulated device. The results showed that in manually performed transfers, even after standardisation of the protocol, there was a large variation in injection speed. Conversely, the pump-regulated device generated a consistent and reproducible injection speed [51]. However, this consistency and reproducibility did not appear to translate into improved reproductive outcomes. In a study including 599 patients randomly assigned to pump or manual transfer, although the pump generated a significantly smaller variance of the positioning of the embryo(s) into the uterine cavity, this did not translate into an overall increased pregnancy rate (21% versus 17%, respectively) [52]. This may be in part due to the possible lack of significance in achieving a precise location for embryo transfer as discussed in the previous section. In general, most practitioners would inject the embryo slowly in the belief that it would avoid injecting the embryo into the fallopian tube despite the lack of evidence to confirm it.

Should we delay the removal of the catheter following transfer?

This has been common practice and continues to be even now in many units. It has, to date, been addressed by a single RCT of 100 women who underwent transfer of at least two optimal embryos. The women were prospectively randomised to two groups: a slow catheter withdrawal group and a delayed catheter withdrawal group (30 s delay). The pregnancy rates in the two groups were 60.8% and 69.4%, respectively, with no significant differences [53]. These results were supported by those of a retrospective study almost a decade later, including 218 women where again no difference was noted

between immediate versus delayed catheter removal techniques [54]. It appears, therefore, that there is no benefit in delaying the withdrawal of the transfer catheter following injection of the embryo.

What about difficult embryo transfers?

This is something that may concern both clinicians and patients alike. One systematic review including a pooled analysis of five studies did, in fact, demonstrate a lower clinical pregnancy rate following non-easy embryo transfers (RR = 0.75; 95% CI 0.66–0.86). This included three studies describing subjectively difficult transfers (RR = 0.67; 95% CI 0.51–0.87) and two studies in which there was a need for additional manoeuvres (RR = 0.78; 95% CI = 0.67–0.91). Interestingly, and rather reassuringly, the presence of blood on the transfer catheter did not appear to affect the clinical pregnancy rates in five studies analysed (RR = 0.96; 95% CI 0.82–1.14) [55].

In our own prospective observational study of 284 women, we also assessed a number of factors relating to the nature and technique of the embryo transfer procedure. Interestingly, we found that women who experienced pain during ET had a significantly lower clinical pregnancy rate than women who did not (42.2% versus 53.8%; $P < 0.05$). Non-pregnant women also had significantly higher pain scores than pregnant women (10.3 versus 6.4; $P = 0.01$). However, pain was a significant predictor of outcome independent of >20 variables including the difficulty of the embryo transfer procedure, which was surprising. In fact, on binary logistic regression analysis, pain ranked fourth as the most important independent predictor for the chance of clinical pregnancy (OR, 0.59; 95%CI 0.37–0.94; $P < 0.05$), following the quality of embryo transferred, uterine contractility and age of the patient [56]. These data suggest that other than difficult embryo transfers, the subjective experience of pain reported by the patients may reflect underlying physiological processes that correlate to reproductive outcomes. More studies investigating both difficult and painful embryo transfers could help explain these correlations and develop effective treatment strategies to improve reproductive outcomes in these women.

Are retained embryos associated with a worse reproductive outcome?

The ASRM Practice Committee evaluated the data on this question and identified 12 relevant studies (secondary outcome of 1 RCT, 10 cohort studies and 1 series). They found that in all but one report, the clinical outcomes of implantation, clinical pregnancy and spontaneous abortion rates were statistically unchanged for patients experiencing embryo retention in their first embryo transfer attempt. The one study that reported a significant adverse outcome described a statistically significant reduction in implantation rate (13% versus 17%; $P < 0.05$) after the re-transfer of 29/584 embryos, although there was no statistical difference in the clinical pregnancy rate [32]. It would appear, therefore, that the immediate re-transfer of retained embryos is unlikely to affect the reproductive outcomes of these women.

Post embryo transfer

Should we recommend bed rest post embryo transfer?

Bed rest post embryo transfer was practiced in the early days of ART and is something patients are often concerned about. In an RCT of 182 women, a 24 h period of bed rest was compared to a 20 min period of bed rest following embryo transfer. The authors found similar pregnancy rates in both groups and, therefore, did not recommend advising prolonged bed rest [57]. Interestingly, a few years later, a similar RCT comparing 24 h bed rest versus 1 h bed rest following embryo transfer reported implantation rates to be significantly reduced in women having 24 h bed rest (9% versus 14.4%, respectively) [58]. Following this study, prolonged bed rest was rarely recommended/practiced.

Further studies started assessing a shorter period of bed rest versus no bed rest at all. One study demonstrated that 30 min of bed rest versus no bed rest after embryo transfer did not improve

Table 1
Summary of 'Good' and 'Fair' evidence regarding the embryo transfer procedure (adopted from the ASRM guideline on embryo transfer).

Variable/Intervention	Recommendation	Grade
Ultrasound guidance	Recommended	A
Soft embryo transfer catheter	Recommended	A
Bed rest	Not recommended	A
Acupuncture	Not recommended	B
Prophylactic antibiotics	Not recommended	B
Removing cervical mucus	Recommended	B
Embryo transfer to central or upper cavity	Recommended	B
Delayed catheter removal	Not recommended	B
Immediate re-transfer of retained embryos	Recommended	B

pregnancy rates (50% versus 46.3%, respectively) [59]. Another study comparing 10 min of bed rest versus no bed rest found the live birth rates to be, in fact, significantly higher in the no bed rest group than in the bed rest group (56.7% versus 41.6%), postulating that there may be negative anatomical/physiological or psychological reasons underlying bed rest with regard to successful implantation [60].

Finally, a systematic review and meta-analysis including 4 RCTs (total n = 757 women) concluded that bed rest following embryo transfer did not improve clinical pregnancy and live birth rates but may, in fact, reduce the implantation rate. The authors concluded that bed rest is not beneficial following embryo transfer and might, in fact, negatively affect the reproductive outcome of ART cycles through various stress and anxiety mechanisms [61].

Should we use therapies such as acupuncture and anti-contractility agents post embryo transfer?

The majority of such interventions are invariably initiated prior to embryo transfer. Therefore, in the absence of specific studies examining their use post transfer alone, data should be extrapolated from the studies that use them both prior to and post the transfer procedure.

With regard to acupuncture, the majority of studies have used acupuncture both prior to and post embryo transfer, with the meta-analyses showing no benefits with regard to reproductive outcomes [4,5]. One RCT, however, by Dieterle et al. (2006) compared the use of acupuncture 30 min and 3 days post embryo transfer demonstrating a clinical pregnancy rate and ongoing pregnancy rate (33.6% and 28.4%, respectively) significantly higher than those of the placebo group (15.6% and 13.8%, respectively) [62]. However, given that this is a single RCT and one of the smallest trials that is at odds with the results of the meta-analyses, the results should be interpreted with caution.

Equally, most trials using Atosiban have initiated the infusion 30 min prior to embryo transfer and continued the infusion for 2 h post transfer [13–15]. Future such studies should, however, investigate the effect of varying regimes on reproductive outcomes, as there are preliminary reports that they may differ with varying dosages and timings [63].

Summary

Embryo transfer is a rate-limiting step in the process of ART treatment. Many factors, some prior to and others during and after the embryo transfer procedure, may affect the outcome. In the present review, we have attempted to systematically outline the available evidence pertaining to this procedure and also highlight areas where future studies could focus on. A summary of the available evidence analysed in the present chapter and in conjunction with the findings of the ASRM Practice Committee is displayed in Table 1.

Practice points

1. Assessment of biological and psychological stress prior to and post ET does not appear to predict reproductive outcomes.
2. Acupuncture at the time of embryo transfer does not appear to improve reproductive outcomes.
3. Removal of cervical mucous at the time of embryo transfer appears to improve reproductive outcomes.
4. A soft versus a hard embryo transfer catheter appears to be associated with improved reproductive outcomes.
5. Transabdominal ultrasound guidance during embryo transfer appears to improve reproductive outcomes, whereas the use of transvaginal or 3D ultrasound does not appear to convey any additional benefit.
6. Delayed embryo catheter removal does not seem to improve reproductive outcomes.
7. Embryo retention and re-transfer does not appear to be associated with a worse reproductive outcome.
8. Bed rest should not be recommended following embryo transfer.

Research agenda

1. Uterine contractility at the time of embryo transfer is correlated to reproductive outcomes, although we are yet to determine how to translate this information into meaningful treatment strategies. Future studies should assess different protocols for agents such as oxytocin antagonists, anticholinergics, prostaglandin synthetase inhibitors and calcium channel blockers in selected group of patients.
2. Evidence from a single RCT does not support the use of prophylactic antibiotics prior to embryo transfer. However, future studies could investigate the use of different antimicrobial agents prior to embryo transfer in selected groups of women, for example those with suspected endometritis.
3. Although most recommendations suggest transferring the embryo to the centre of the uterine cavity (1–2 cm from the fundus), there is heterogeneity in the data available, which may be explained by the fact that the embryos are likely to undergo some physiological migration following transfer. Future studies should assess the location and migration of embryo transfer in conjunction with uterine contractility and associated anti-contractility agents.
4. Difficult and painful embryo transfers appear to be correlated to poor reproductive outcomes. Future studies could investigate the underlying mechanisms behind these adverse outcomes and examine treatment strategies to improve the reproductive outcomes of such patients.

Conflict of interest

The authors have no conflicts of interest with regard to the present article.

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