

The presence of blood in the transfer catheter negatively influences outcome at embryo transfer*

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BACKGROUND: Embryo transfer (ET) influences pregnancy rates in patients undergoing assisted reproduction. Data are conflicting as to which variables affect ET success. This study examines variables that may affect outcome after ET in assisted reproductive technology patients who had high-quality embryos transferred. **METHODS:** Over a 23 month period, 669 consecutive cycles were examined. Only patients having grade I and grade II embryos, or blastocyst transfers, were included in this retrospective analysis. A total of 584 consecutive cycles met study criteria. At the time of ET, the following variables were recorded: aborted first attempt at ET; presence of blood and/or mucus in or on the transfer catheter after ET; ease of ET as judged by provider; need for mock embryo transfer immediately before the actual transfer and retention of embryos in the transfer catheter. These variables were retrospectively analysed for their impact on implantation rate (IR) and clinical pregnancy rate (CPR). **RESULTS:** There were 290 gestations (49.7% CPR). Multiple attempts at ET, subjective difficulty of ET, performance of a sham pass immediately prior to embryo transfer, and presence of mucus on or in the catheter did not affect the CPR or IR. No difference was noted in the mean age of patients having or lacking any of these factors. There was a significant association between the presence of blood on or in the catheter and decreased IR ($P = 0.015$) and CPR ($P = 0.004$). Retained embryos also decreased IR ($P = 0.03$). Multivariable analysis confirmed that the presence of blood on the transfer catheter was the most important of these transfer characteristics in predicting IR ($P = 0.042$) and CPR ($P = 0.018$). **CONCLUSIONS:** These results suggest that when only high-grade embryos or blastocysts are transferred, the presence of blood on the catheter is associated with decreased IR and CPR in assisted reproduction.

Key words: blood/embryo transfer technique/implantation/pregnancy rate/retained embryos

Introduction

Clinical pregnancy rates (CPR) for patients undergoing assisted reproductive technologies (ART) are influenced by embryo transfer (ET) technique, as optimal CPR cannot be achieved without correct placement of embryos into the uterine cavity at ET (Nabi *et al.*, 1997; Awonuga *et al.*, 1998; Karande *et al.*, 1999; Hearns-Stokes *et al.*, 2000). 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 CPR after ET, there exists a significant inconsistency with respect to presence of blood or mucus on the catheter, retention of embryos in the transfer catheter, multiple attempts at ET (aborted ET), ease of ET, and requirement of a mock ET

immediately preceding ET, and their association with implantation rate (IR) and CPR. It is possible that this is because embryo quality was poorly controlled for in these previous studies.

Edwards *et al.* (1984) did not observe a statistically significant effect of catheter contamination with blood on CPR. These results were supported by Visser *et al.* (1993). However, Goudas *et al.* (1998) and Awonuga *et al.* (1998) found that the presence of blood on the transfer catheter after ET was associated with decreased IR and CPR. Visser *et al.* (1993) observed that retention of embryos in the transfer catheter significantly reduced the CPR, and that catheters contaminated with blood and cervical mucus indirectly contributed to this effect by increasing the incidence of failed first attempts at ET. In contrast, Nabi *et al.* (1997) and Goudas *et al.* (1998) observed that retention of embryos in the transfer catheter did not significantly reduce CPR. More recent studies have also found that there was no significant difference in the

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CPR between transfer procedures in which all embryos were transferred at the first attempt and those which required multiple attempts (Wood *et al.*, 1985; Egan *et al.*, 1990; Nabi *et al.*, 1997).

Although the presence of mucus in the transfer catheter has been associated with an increased incidence of retained embryos at ET, several studies have not demonstrated a significant effect on CPR (Visser *et al.*, 1993; Awonuga *et al.*, 1998). The presence of both blood and mucus has been implicated in difficult transfers (Awonuga *et al.*, 1998). Some studies report that difficulty of the ET does not affect pregnancy outcome (Wood *et al.*, 1985; Egan *et al.*, 1990; Tur-Kaspa *et al.*, 1998), while others suggest that difficult transfers adversely affect pregnancy outcome (Leeton *et al.*, 1982; Mansour *et al.*, 1990).

In this study, we have evaluated the effect of several factors on pregnancy outcome after ET. Given the strong influence of embryo quality and ultrasound guidance on pregnancy outcome, we have controlled for these variables. We also examined the mean age for each characteristic to make sure that age did not impact outcome.

Materials and methods

Patients

The records of 669 consecutive, ultrasound-guided embryo transfers performed between January 1, 1998 and November 30, 1999 at the assisted reproduction programme of Walter Reed Army Medical Center were retrospectively reviewed. Patients aged >43 years, those with serum FSH levels ≥ 12 IU/ml on cycle day 3, and those who received cryopreserved cleaving embryos or blastocysts were excluded from the study. Only patients who underwent transfer of at least one high quality embryo (grade I or grade II) or blastocyst were included in the study. A total of 78 cycles was excluded for lack of high-grade embryos or blastocysts, six cycles were excluded for incomplete data and one patient was aged >43 years. As a result, 584 cycles met study criteria. This study was reviewed and approved by the Walter Reed Army Medical Center Clinical Investigation Committee.

Experimental design

Stimulation protocols were individualized to the patient but, in general, patients aged >35 years underwent a microdose flare GnRH agonist protocol (Leondires *et al.*, 1999) and initially received an oral contraceptive containing levonorgestrel and ethinyl estradiol. After cessation of oral contraceptives, patients received either Lupron 20 μg or 40 μg twice daily and gonadotrophins (FSH or a combination of FSH and hMG). Younger women or patients at high risk for hyperstimulation underwent a luteal suppression GnRH agonist protocol with Lupron (TAP Pharmaceuticals, USA) 0.5 mg/day starting in the mid-luteal phase until ovarian quiescence was achieved (serum estradiol <35 pg/ml). The dosage of lupron was then reduced to 0.25 mg s.c. daily and gonadotrophins (FSH or a combination of FSH and hMG) were started. All cycles were monitored with serial follicular ultrasound measurements and serum estradiol levels. hCG was given when the lead follicle had an average diameter of 18 mm and the majority of the cohort measured ≥ 14 mm. Oocyte retrieval took place 35 h after the hCG injection.

Semen preparation, oocyte insemination and injection, embryo culture and embryo scoring were performed using our laboratory's

standard technique (Scott *et al.*, 2000). Providers adhered to ASRM guidelines for the number of embryos transferred (Society for Assisted Reproductive Technology, 1999). Cleaving embryos were transferred at day 2 or day 3 and blastocysts were transferred at day 5. The medium used during the ET was the same as that in which the embryos were being cultured. All embryo transfers were performed with the Wallace catheter (SIMS Portex Ltd, UK), and visualized with transabdominal ultrasound (Ultramark 4 with a 5 MHz transducer). The patients started luteal supplementation with progesterone in oil 50 mg i.m. on the night of retrieval and continued until a negative pregnancy test occurred at 2 weeks post-retrieval or until 8 weeks gestation.

Prior to starting an ART cycle, all patients underwent a mock embryo transfer to determine the direction of the endocervical canal and depth of the uterine cavity. If it was anticipated that the transfer would be very difficult on the basis of the pre-cycle mock transfer or other historical reason, the transferring physician might elect to perform a mock transfer immediately prior to the actual procedure. At the discretion of the provider, once cavity placement was assured, the outer sheath could be left, the inner sheath removed and an inner sheath with embryos loaded could then be placed through the in-situ outer sheath. The embryos were thereby protected from prolonged exposure to the environment and to a possibly traumatic transfer. Patients underwent transfer in the dorsal lithotomy position with a full bladder. A sterile, preheated, bivalve speculum was inserted into the vagina to expose the cervix. The cervix was cleaned with sterile cotton swabs and embryo culture media. Endocervical mucus was removed at the discretion of the transfer physician, but, in general, cervical lavage with media and aspiration with a pipette were not used in this programme. Uterine orientation and the endometrial cavity were identified with abdominal ultrasound prior to the transfer. The embryologist used a sterile 1 ml syringe to flush 1 ml of growth media through the catheter, leaving 0.01 ml intraluminally. The embryos were suctioned from the growth media followed by 5 μl of air. The embryologist passed the loaded catheter to the physician, who carefully deposited the embryos, ~1.0–1.5 cm from the uterine fundus. An air bubble was visualized on ultrasound after deposition of embryos into the endometrial cavity. The plunger of the catheter remained depressed from the point during which the embryos were deposited until the catheter was passed to the embryologist. The embryologist flushed the catheter with growth media and microscopically examined it for the presence of blood, mucus, and retained embryos. Any embryos retained were immediately reloaded by the embryologist and a second transfer performed. The physician who performed the transfer immediately scored the transfer as easy, moderate or difficult, recorded the number of passes required to successfully perform the transfer, whether a mock embryo transfer was performed immediately preceding the ET, and if the transfer was visualized on abdominal ultrasound. After consultation with the embryologist, the physician recorded whether embryos were retained in the transfer catheter, and if blood or mucus were present on or in the catheter.

Statistical analysis

The principal outcome variables were the presence of a clinical pregnancy, defined as a gestational sac visualized on ultrasound at 6–8 weeks gestation, and IR defined as the number of gestational sacs seen on ultrasound divided by the number of cleaving embryos or blastocysts transferred.

Univariable analysis was used to determine whether the presence of blood or mucus on the transfer catheter, ease of ET, retention of embryos in the transfer catheter, requirement of multiple attempts to achieve successful ET, or requirement of a mock ET immediately

Table I. Clinical pregnancy rate (CPR) and implantation rate (IR), age and mean number of embryos transferred (ET)

Parameter	Age (years)	n (%)	No. of ET	P	CPR (%)	P	IR (%)	P
No blood	34.1 ± 4.5	524 (89.7)	2.93	0.5	51.7	0.004	31.3	0.015
Blood	34.9 ± 4.2	60 (10.3)	2.83		31.7		19.5	
Not retained	34.2 ± 4.4	555 (95)	2.90	0.03	50.0	0.45	31.0	0.03
Retained	35.4 ± 4.5	29 (5)	3.30		41.4	NS	17.0	
No mucus	34.2 ± 4.5	463 (79.3)	2.93	0.9 (NS)	48.8	0.48 (NS)	30.0	0.8 (NS)
Mucus	34.3 ± 4.3	121 (20.7)	2.91		52.9		31.0	
No sham	34.2 ± 4.4	545 (93.3)	2.95	0.02	50.6	0.1 (NS)	30.0	0.7 (NS)
Sham	33.5 ± 4.6	39 (6.7)	2.54		35.9		28.0	
Not aborted	34.2 ± 4.5	532 (91.1)	2.91	0.5 (NS)	50.2	0.47 (NS)	30.0	0.4 (NS)
Aborted	34.2 ± 3.8	52 (8.9)	3.02		44.2		26.0	

NS = not significant.

Table II. Clinical pregnancy rate (CPR) and implantation rate (IR) and mean number of embryos transferred (ET), according to difficulty of the transfer

	n (%)	Mean no. of ET	IR	CPR (%)
Easy	445 (76)	2.9	0.31	51.2
Moderate	84 (14)	2.9	0.29	47.6
Difficult	54 (10)	2.9	0.23	40.7
P		0.91 (NS)	0.29 (NS)	0.32 (NS)

NS = not significant.

preceding ET were associated with IR or CPR. IR data were analysed using Student's *t*-test for all but the 'difficulty' category, which were evaluated using one-way analysis of variance. CPR were analysed using Fisher's exact test with the exception of the 'difficulty' category which was evaluated using the χ^2 -test. Multivariable analysis was used to control for confounding in the variables that were studied. Specifically, IR was evaluated using analysis of covariance (ANCOVA) to allow the use of a combination of continuous and categorical variables as the independent variables. Since CPR is a categorical variable, logistic regression was used to quantify the association of this dependent variable with the independent variables.

Statistical Package for the Social Sciences (SPSS), version 10.0 was used for all the statistical calculations in this study. $P \leq 0.05$ was considered statistically significant.

Results

The mean ± SD age of all patients was 34.2 ± 4.4 (range 22–43). As would be expected, there was a significant difference in age among between who achieved pregnancy (33.40 ± 0.24) and those who did not (34.98 ± 0.26, $P < 0.0005$). The overall CPR and IR were 49.1 and 29.9% respectively.

A total of 10 providers performed the embryo transfers and this analysis is a report of all the practitioners' results. An attempt to evaluate individual physicians' outcomes did not reveal a statistically significant difference among the providers with regard to CPR, IR, embryo retention rate, and the presence of blood or mucus on the catheter. There were differences in perceived difficulty of transfer ($P < 0.0005$) and rate of aborted transfers ($P < 0.05$) among the providers, possibly reflective of the more experienced physicians tending to perform the more challenging procedures.

Table III. Strength of the association between implantation rate (dependent variable) and blood on or in transfer catheter, retained embryos at transfer, difficulty of transfer, mucus on or in transfer catheter, need for a sham transfer prior to actual procedure or aborted initial attempt at transfer, using analysis of covariance

Parameter	F	P
Blood on/in catheter	3.7	0.04
Retained embryo at transfer	3.2	0.07 (NS)
Difficulty of transfer	0.3	0.56 (NS)
Mucus in catheter	0.7	0.38 (NS)
Sham at transfer	0.3	0.62 (NS)
Aborted transfer	0.01	0.89 (NS)

NS = not significant.

VF accounted for 437 and ICSI for 147 of the embryo transfers that took place. There were no differences in the outcome variables with regard to type of fertilization procedure performed.

Blood was noted on the transfer catheter after 60 embryo transfers (10.3%). As previously noted, age was not a factor as there was not a statistically significant difference in the mean age of patients in the group with the presence of blood on the transfer catheter after ET (34.9 ± 4.2) compared with the group without the presence of blood (34.1 ± 4.5, $P = 0.23$, not significant). Overall, CPR were substantially affected by the presence of blood on the catheter, with a 51.7% chance of a gestational sac with fetal pole and cardiac activity if no blood was found, compared to 31.7% if blood was discovered (Table I, $P = 0.004$). The IR was also affected by the presence of blood on the catheter. The mean IR for those in which no blood was found was 31.3% compared to 19.5% with blood (Table I, $P = 0.015$).

Of the 584 cycles analysed, embryos were retained in the catheter after ET in 29 ET (5.0%). While CPR was not different, the overall IR was 31% in ET where embryos were not retained and 17% for those where embryos were retained (Table I, $P = 0.03$). Interestingly, there were a significantly greater number of embryos transferred in the group that had retained embryos. This explains why the CPR in both groups remained comparable but it is uncertain why a greater number of embryos were transferred. The decision as to number of embryos to transfer is obviously made before the retention event so they should not influence each other in any way.

Table IV. Strength of the association between CPR (dependent variable) and blood on or in transfer catheter, retained embryos at transfer, difficulty of transfer, mucus on or in transfer catheter, need for a sham transfer prior to actual procedure or aborted initial attempt at transfer, using logistic regression

Parameter	<i>P</i>
Blood on/in catheter	0.01
Retained embryo at transfer	0.49 (NS)
Difficulty of transfer	0.80 (NS)
Mucus in catheter	0.18 (NS)
Sham at transfer	0.32 (NS)
Aborted transfer	0.94 (NS)

NS = not significant.

There were no significant differences in IR or CPR for presence of mucus on the transfer catheter, ease of ET as judged by the provider, occurrence of aborted attempts to achieve successful ET, or requirement of a mock ET immediately preceding ET (Tables I, II).

Of course it is unlikely that these are completely independent variables. Using IR and CPR as the dependent variables, the aforementioned parameters were analysed using multivariable statistics to assess their relative contribution to outcome. As is seen in Table III, the presence of blood on or in the transfer catheter is the only parameter that is significantly associated with IR when ANCOVA is used to assess the relationship. Retained embryos demonstrate a trend toward being significant but do not actually achieve significance. In Table IV, the results of logistic regression again show that blood significantly compromises CPR whereas the other parameters do not appear to influence outcome.

Discussion

Despite the importance of ET, relatively few studies have evaluated ET technique and the results of these studies are conflicting. Data suggest that transfer of high-grade embryos or blastocysts and ET performed under ultrasound guidance are associated with increased CPR in IVF (Scott *et al.*, 1991; Roseboom *et al.*, 1995; Gardner *et al.*, 1998). It is possible that the conflicting reports in the literature with regard to the importance of other transfer parameters may be explained in part by differences in embryo quality. In an attempt to clarify which variables negatively influence outcome in assisted reproduction, we have evaluated the effect of several variables on success at ET while controlling for embryo quality and ultrasound guidance. Our results suggest that the presence of blood on the catheter during ET negatively impacts success at assisted reproduction.

Although Nabi *et al.* (1997) and Goudas *et al.* (1998) observed that retention of embryos in the catheter did not significantly reduce CPR, we found that retention of embryos was associated with a significant reduction in IR. The IR was 31% for ET in which embryos were not retained and 17% for those in which embryos were retained ($P = 0.03$). As previously noted, CPR is not affected, largely because a greater number of embryos were transferred. There is no easy

explanation for why such a significant discrepancy in number of embryos transferred exists.

Although the presence of mucus on the transfer catheter did not affect CPR or IR in our study, it was associated with an increased incidence of retained embryos (data not shown). In agreement with the results of Awonuga *et al.* (1998), we found that embryos were more likely to be retained when the transfer catheter was contaminated with mucus. Retention of embryos may indicate mechanical blockage of the catheter opening (Leeton *et al.*, 1982; Egan *et al.*, 1990; Visser *et al.*, 1993) and mucus may cause embryos to adhere to the catheter when it is withdrawn (Awonuga *et al.*, 1998). Our findings lend support to the suggestion of Awonuga *et al.* (1998) to remove significant cervical mucus prior to ET in an effort to decrease the incidence of retained embryos. Nevertheless, the presence of mucus does not appear to be a significant factor in ET. The subjective scoring of transfers as difficult did not reduce CPR or IR. The difficulty or ease with which a provider scored an ET is troublesome to assess because of its subjective nature but did not appear to affect outcome. Nevertheless, assessment (and self-assessment) of the provider's ET technique and skill acquisition has been an issue of interest to our programme (Hearns-Stokes *et al.*, 2000; Papageorgiou *et al.*, 2001). Consciousness of a greater than average number of 'difficult' transfers might induce a provider to retrain in transfer technique.

Our observations were similar to those of Wood *et al.* (1985), Egan *et al.* (1990) and Nabi *et al.* (1997) who also reported that the CPR was not influenced by the number of attempts required to replace embryos, and ET that required multiple attempts were not associated with compromised CPR or IR. In our programme, embryos were returned to the laboratory if the ET could not be completed within 2 min and a mock ET was performed to determine whether cervical obstruction existed, direction of endocervical canal, and depth and direction of endometrial cavity. We have empirically begun this protocol in an effort to minimize the potential for damage or loss of embryos, or trauma to the endometrium.

The results of this retrospective review highlight two possible areas for intervention. If, as was earlier suggested, some women have evidence of blood on the transfer catheter despite no obvious trauma, an endometrial problem may exist. We did not attempt to correlate ultrasound lining characteristics with the incidence of blood on the catheter but this may be worthwhile to assess. This subpopulation of women may fall in the category of an endometrial factor. Although treatment modalities in this area are at present suboptimal, ongoing research will in the future offer practical and effective interventions.

It is impossible to know at this time if there is endometrial bleeding or if cervical bleeding is a marker for significant endometrial trauma due to transfer technique. Cervical trauma may be the more common cause for blood on the catheter tip. There is also the possibility that a subclinical infection, such as bacterial vaginosis, might lead to cervical friability. It is possibly a combination of all of these factors. Regardless of the source, it is clear from this study that blood on the catheter is a marker for reduced success in assisted reproduction. As IVF

becomes more successful, it would be prudent to identify the cause for this small but discrete subset of individuals. Possible causes include bleeding disorders, medications or endometrial surface abnormalities.

As with difficult transfers, a large percentage of ET associated with the presence of blood might suggest that a provider reassess ET technique and reassess their training. Much of the previously cited research indicates that a steep learning curve exists for ET. Goudas *et al.* (1998) suggested that an emphasis on atraumatic ET technique with prevention of bleeding at time of ET should contribute to improved pregnancy outcome in IVF. In our programme, providers are required to perform a significant number of mock transfers and intrauterine inseminations using a soft catheter before they are allowed to transfer embryos. Developing the fine motor skills integral to such a nuanced procedure is critical to success. Moreover, developing the confidence to 'feel' the endocervical pathway rather than forcing the catheter cannot help but decrease traumatic delivery of the embryos to the endometrial cavity.

In summary, avoidance of blood on the transfer catheter and perhaps retained embryos are key to good CPR and IR. Our results support the conclusion that ET technique plays a major role in assisted reproduction outcome. Attention to detail, atraumatic transfers, and frequent assessment of providers' technique should positively impact outcome in assisted reproduction.

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