Does the Presence of Blood in the Catheter or the Degree of Difficulty of Embryo Transfer Affect Live Birth?

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Abstract

The technique used for embryo transfer (ET) can affect implantation. Prior research that evaluated the effect of postprocedural blood of the transfer catheter tip have yielded mixed results, and it is unclear whether this is actually a marker of difficulty of the transfer. Our objective was to estimate the effect of blood at the time of ET and the difficulty of ET on live birth rates (LBR). This retrospective cohort study utilized generalized estimating equations (GEEs) with nesting for repeated cycles for all analyses. Univariate modeling was performed and a final multivariate (adjusted) GEE model accounted for all significant confounders. Embryo transfers were subjectively graded (easy, medium, or hard) by a physician at the time of transfer. The presence of blood at ET was associated with more difficult ETs, retained embryos, and presence of mucous in the catheter. In the univariate analysis, ET with blood was not associated with live birth, while the degree of difficulty for ET had a negative impact on LBR. In the final multivariate GEE model, which accounts for repeated cycles from a patient, the only factors associated with an increased LBR were the degree of difficulty of the ET, female age, and blastocyst transfer. After controlling for confounding variables, the presence of blood in the transfer catheter was not associated with the likelihood of pregnancy and thus was not an independent predictor of cycle outcome. This indicates that the difficulty of the transfer itself was a strong negative predictor of pregnancy.

Keywords

ART, blood on catheter, embryo transfer, difficult transfer, degree of difficulty

Introduction

Procedural technique at the time of embryo transfer (ET) is important for providing optimal conditions for implantation to proceed successfully. Studies demonstrate higher pregnancy rates with ultrasound guidance,^{1,2} bladder distension,³ mock transfer⁴ or embryo afterloading,⁵ use of soft-tip catheters,⁶ and site of embryo deposition within the uterus.⁷⁻⁹ Two additional key parameters are catheter tip contamination with blood and the difficulty of the transfer.¹⁰

Studies are controversial regarding the postprocedural presence of blood on the transfer catheter tip, with many demonstrating a significant decrease in the likelihood of achieving pregnancy,¹¹⁻¹⁶ although others have found no effect.^{17,18} The presence of blood in the catheter after transfer is generally thought to represent cervical or endometrial bleeding, which may interfere with implantation. This bleeding may be due to a traumatic transfer and the presence of blood and the difficulty of transfer may be closely related.

Difficult transfers have been described as ETs that are subjectively rated as difficult, take additional time or effort, require firmer catheters, or require instrumentation such as a

tenaculum. The current literature is controversial regarding the impact on cycle success, with some studies demonstrating no effect of transfer difficulty in experienced hands^{19,20} and others showing a significantly lower pregnancy rate.^{4,21-25}

Given the conflicting evidence regarding the association of blood on the catheter in conjunction with the difficulty of ET with cycle outcome, we sought to further assess the association of these factors with success after in vitro fertilization (IVF).

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Although the afterload method of ET may have a favorable effect on pregnancy, the influence of blood and the degree of difficulty have not been reported using that method. The objective of this study was to estimate the effect of blood at the time of ET and the difficulty of ET on live birth after IVF.

Materials and Methods

Study Design

This study was a retrospective cohort analysis of all assisted reproductive technology (ART) cycles with ET from January 2012 to December 2013 at Walter Reed National Military Medical Center, Bethesda, Maryland. Inclusion criteria were ET of fresh or vitrified autologous embryos during the study period. The ART cycles where no ET occurred were excluded. The primary outcome was live birth, defined as a live born infant delivered after 24 weeks' gestation. Secondary outcomes included clinical pregnancy (fetal cardiac activity), miscarriage, and blood on the catheter during ET. This study was approved by the institutional review board of Walter Reed National Military Medical Center.

Assisted Reproductive Technology Protocols

Patients were pretreated with oral contraceptive pills and underwent a gonadotropin-releasing hormone (GnRH) microdose flare, long luteal, or GnRH antagonist protocol as previously described.²⁶ Patients were monitored with serial serum estradiol levels and transvaginal ultrasound measurements of follicle size and received a single intramuscular injection of human chorionic gonadotropin when at least 2 follicles were 18 to 20 mm in size. Transvaginal oocyte retrieval under ultrasound guidance was performed 35 hours later, and conventional IVF or intracytoplasmic sperm injection was performed as indicated. Luteal support was provided with intramuscular progesterone (50 mg daily) beginning on the day of retrieval or intravaginal progesterone (200 mg 6 times per day) beginning the day following retrieval. Luteal support was continued until 10 weeks' gestation.

All ETs were performed with abdominal ultrasound guidance utilizing either 18 or 23 mm Wallace embryo replacement catheters (Smiths Medical, London, United Kingdom). Embryo transfer was completed utilizing the afterload technique.⁵ First, a double catheter was inserted into the cervix and advanced to just past the internal cervical os under ultrasound guidance. After the passage was confirmed, the embryologist loaded the embryos into an identical inner catheter and the mock inner catheter in the uterus was removed, leaving the outer catheter in place. The inner catheter with the embryos was then placed through the outer catheter and advanced slowly under ultrasonic guidance until it reached a depth of 1 cm from the fundal endometrium. Next, the embryos were transferred. Subsequently, the inner catheter was removed and taken back to the laboratory for microscopic evaluation of blood or mucous on or in the transfer catheter. Additionally, the embryologist evaluated for the presence of a retained embryo in the transfer catheter. During this

time, the transferring physician continued to hold the outer catheter in place while awaiting the embryologist's assessment. The outer catheter was removed if there were no retained embryos. However, if a retained embryo was discovered, it was reloaded into an inner catheter and retransferred through the outer sheath. The transferring physician recorded the degree of difficulty of the ET as an assessment of easy, medium, or hard prior to being told if there was microscopic blood or mucous on the catheter. An "easy" transfer describes one done without any difficulty or additional instrumentation. A "hard" transfer describes one in which additional time or techniques (ie, tenaculum use, a rigid transfer catheter) were necessary to complete passage of the catheter into the endometrium. A transfer was classified as "medium" if it did not meet criteria for either easy or hard transfers. All transferring physicians during this time period were consistent regarding the difficulty rating. If a fellow performed the transfer, the difficulty rating was discussed and agreed upon with the supervising attending. Serum quantitative human chorionic gonadotrophin testing was performed 14 and 16 days after oocyte retrieval. Transvaginal ultrasound was performed approximately 3 weeks later to confirm an intrauterine pregnancy.

Statistics

Data for the cohort were assessed with mean $\pm$ standard deviation (SD) for parametric or median with ranges for nonparametric distributions. The primary data analyses were performed utilizing generalized estimating equations (GEEs) modeling with nesting for repeated cycles within a patient. Continuous variables and dichotomous variables were assessed with the GEE models based on the type of data distribution. Unadjusted univariate GEE models were utilized to assess for factors associated with the presence of blood at ET and for factors associated with live birth. Variables found to be statistically significant in unadjusted GEE modeling were then included in adjusted multivariate models. Adjusted GEE modeling with nesting for repeated cycles within a patient and with the variables age, difficulty of ET, blastocyst transfer, the number of embryos transferred, and blood on the transfer catheter along with the interactions of these variables was utilized to further evaluate for variables associated with live birth. The χ^2 was used to compare IVF cycle outcomes and live births based on the presence/absence of blood as well as difficulty of the transfer. *P* values $<.05$ were considered statistically significant. Statistical analysis was performed using SPSS software (version 22, IBM, Armonk, New York).

Results

In this study, 784 patients undergoing 922 ET cycles were included. The mean $\pm$ SD patient's age was 34 ± 5 years. There were 560 blastocyst transfers and 362 cleavage stage transfers. There were 126 frozen-thawed ETs and 796 fresh embryos transfers. Of all transfers, 139 (14.8%) were undergoing their first cycle at our facility and the remainders were

Table 1. Baseline and Cycle Stimulation Comparisons Between the 2 Groups.

Outcome	No Blood at ET	Blood at ET	P Value
Presence of mucous on catheter	11.1%	28.9%	<.001
Retained embryos	1.6%	4.5%	<.001
Age, years	35 (24-42)	34 (22-42)	.16
Parity	2 (0-4)	2 (0-4)	.82
Day 3 FSH, mIU/mL	7 (2-14)	7 (2-12)	.54
Days of stimulation	11.2 ± 1.5	11.2 ± 1.7	.45
Endometrial thickness, mm	11 (6-18)	11 (7-16)	.59
Embryos transferred	1.8 (1-4)	1.8 (1-5)	.99
Blastocyst transfer	55.0%	56.1%	.86
FET	18.3%	13.0%	.77

Abbreviation: ET, embryo transfer; FSH, follicle stimulating hormone; FET, frozen embryo transfer.

Table 2. Comparison of Pregnancy Outcomes in Patients Without and With Blood on the Catheter at Embryo Transfer (ET).

Outcome	No Blood at ET (%)	Blood at ET (%)	P Value (χ^2)	OR (95% CI) From Unadjusted GEE Model
Live birth	41.2	34.8	.15	0.93 (0.86-1.02)
Clinical pregnancy	49.2	40.5	.054	0.91 (0.84-0.99)
Positive hCG	60.8	49.3	.007	0.89 (0.82-0.97)
Spontaneous abortion	13.3	11.5	.72	0.97 (0.93-1.01)
Chemical pregnancy	19.1	17.9	.87	0.97 (0.92-1.02)
Ectopic pregnancy	1.3	0	.60	0.95 (0.90-1.05)

Abbreviations: CI, confidence interval; hCG, human chorionic gonadotrophin; GEE, generalized estimating equations; OR, odds ratio.

undergoing repeat cycles. There were 764 (82.8%) transfers without blood in the catheter and 158 (17.2%) transfers with blood in the catheter. Patient baseline and cycle stimulation characteristics were similar between those with and without the presence of blood in the catheter (Table 1). The 2 groups also had similar clinical pregnancy and live birth outcomes in both χ^2 and GEE models (Table 2).

There were 838 easy transfers, 69 medium difficulty transfers, and 15 difficult transfers as recorded by the transferring physician. Blood at the time of ET occurred in 15% of easy transfers, 33% of medium transfers, and 40% of difficult transfers ($P < .001$).

Variables associated with the presence of blood at ET included medium difficulty ETs (odds ratio [OR]: 2.96, 95% confidence interval [CI]: 1.80-4.86), hard difficulty ET (OR: 4.57, 95% CI: 1.34-85.67), the presence of a retained embryo (OR: 2.13, 95% CI: 1.8-4.86), and the presence of mucous on the catheter (OR: 1.76, 95% CI: 1.46-2.08). Stage of ET, patient's age, the thickness of the endometrium, and the endometrial grade were not associated with blood at ET. In the adjusted model controlling for confounding variables, medium difficulty ETs (OR: 2.48, 95% CI: 1.56-8.55), hard ETs (OR:

Table 3. Pregnancy and Live Birth Rates Based on Degree of Transfer Difficulty.

Variable	n	Pregnancy Rate (%)	Live Birth Rate (%)
Easy transfers	838	48.7	41.2
Medium transfer	69	34.7	27.5
Hard transfer	15	35.7	26.6

4.37, 95% CI: 1.67-83.81), the presence of a retained embryo (OR: 2.31, 95% CI: 1.32-4.07), and the presence of mucous on the catheter (OR: 1.76, 95% CI: 1.46-2.08) remained positively associated with having blood at the time of ET.

When controlling for these factors in the model, the presence of blood was not significantly associated with cycle outcomes (Table 2). Transfers without the presence of blood in the catheter had a 41.2% live birth rate versus 34.8% for transfers with blood ($P = 0.15$). The χ^2 analysis also demonstrated that easy transfers had the highest live birth rate (41.2%) versus medium transfers (27.5%) and hard transfers (26.6%; $P = .006$) (see Table 3). Easy transfers had a similar live birth rate with or without the presence of blood on the catheter (easy + no blood: 41.7% vs easy + blood: 38.7%, $P = .59$).

In univariate GEE models, variables associated with live birth included the patient's age, the difficulty of the ET, blastocyst transfer, and the number of embryos transferred (Table 4). Endometrial grade, retained embryos at transfer, and mucous and blood on the catheter at ET were not associated with live birth. In the adjusted GEE model controlling for confounding variables, easy ET (OR: 1.87, 95% CI: 1.09-3.21), patient's age (OR: 0.92, 95% CI: 0.88-0.96), the number of embryos transferred (OR: 1.32, 95% CI: 1.05-1.70), and blastocyst transfer (OR: 1.53, 95% CI: 1.28-1.84) remained associated with live birth (Table 4). When controlling for the difficulty of ET in the adjusted model, the P value for blood on the catheter during ET being associated with live birth was .53 compared to .12 when not controlling for the difficulty of ET (Table 3).

Discussion

More difficult ETs were independently associated with a lower likelihood of ongoing pregnancy, whereas the presence of blood on the catheter tip was not. These results were similar to studies that found no effect of blood on cycle outcome^{17,27} and a significant negative effect of transfer difficulty,^{20,27} as well as a recent meta-analysis demonstrating similar results for both blood and transfer difficulty.¹⁸ However, the present results differ from several other studies, including a large retrospective study that reported a decrease in clinical pregnancy after ETs with blood on the catheter.¹³

The study by Tiras et al only reported on transfer difficulty within the subgroup having severe blood on the catheter tip, in which difficult transfers negatively impacted cycle outcome. However, the authors did not assess for correlation between transfer difficulty and the presence of

Table 4. Unadjusted and Adjusted GEE Models for Variables Associated With Live Birth.

Variable	P Value	Unadjusted GEE, OR (95% CI)	P Value	Adjusted GEE, OR (95% CI)
Blood at transfer	.12	0.93 (0.86-1.02)	.53	1.12 (0.77-1.63)
Easy transfer	.006	1.98 (1.21-3.23)	.02	R 1.87 (1.09-3.21)
Age	<.00001	0.92 (0.89-0.95)	<.00001	0.92 (0.88-0.96)
Blastocyst transfer	.00008	2.32 (3.08-1.75)	<.00001	1.53 (1.28-1.84)
Embryos transferred (n)	.01	1.34 (1.06-1.70)	.02	1.32 (1.05-1.70)
Mucous at transfer	.78	1.00 (0.94-1.07)	Not included	Not included
Retained embryo at transfer	.77	0.97 (0.80-1.18)	Not included	Not included
Peak endometrial thickness	.14	1.00 (0.99-1.01)	Not included	Not included
Frozen embryo transfer	.55	1.12 (0.76-1.66)	Not included	Not included

Abbreviations: GEE, generalized estimating equations; OR, odds ratio; CI, confidence interval.

blood in the entire cohort, thus they could not control for an interaction between these 2 variables.¹³ It is plausible that severe blood from active bleeding may impact pregnancy rate differently than a small amount of blood on the catheter; however, it is difficult to compare the quantity of blood reported since Tiras et al did not define “severe” blood.^{13,18,28}

Our study also differs from previous studies by the day of ET and the method of ET. The current body of literature largely assesses cleavage stage ETs, whereas more than half of our ETs occurred on day 5. Given the increasing number of blastocyst transfers and the changes in the endometrium that occur with 2 to 3 additional days of development, it is important to reassess the prognostic significance of transfer parameters on cycle success. Thus, because of the higher likelihood of pregnancy following a blastocyst ET, any reduction in pregnancy is more likely to be detected. We found that neither blood nor mucous on the catheter tip was associated with live birth in a population comprised of both cleavage stage and blastocyst transfers. Furthermore, since the afterload method uses a second inner catheter through which embryos are loaded, findings and significance of blood or mucous might differ significantly when compared to direct insertion methods. Notably, our own findings using direct insertion suggested a negative correlation with blood,^{13,16,18,29} but this effect was not observed when the afterload method was used.

The present study has several strengths in addition to the large number of blastocyst transfers. The utilization of GEE modeling with nesting allowed analysis of all cycles within the study period because it accounts for repeated cycles within a patient. Furthermore, univariate analysis identified factors associated with the presence of blood and live birth that were subsequently included in an adjusted multivariate model, thus, we were able to control for interactions between the variables. Since we demonstrated a significant association between blood on the catheter and the difficulty of the ET, it is important to control for the interaction between these variables. When using the adjusted model that accounted for difficulty of transfer, the presence of blood in the transfer catheter was not associated with live birth. Furthermore, we report on the main outcome of live birth, which is largely missing in the current body of literature, as noted by Phillips et al in their recent meta-analysis.¹⁸

The current study is limited by its retrospective design, which could introduce uncontrolled bias, and the lack of data regarding uterine anomalies, fibroids, cervical stenosis, and uterine flexion. These uterine factors may significantly influence the degree of difficulty of ET. All patients in the study had a normal uterine cavity on saline ultrasound within a year prior to their IVF cycle, but we were unable to control for uterine factors not related to the uterine cavity itself. An additional weakness is the subjective nature of the measure of transfer difficulty. The ETs were performed by a heterogeneous group of fellows and attending providers, which may be a limitation but could also make the study more generalizable.

In conclusion, ET is a delicate procedure that is an integral part of assisted reproduction. Although many other factors also impact ultimate success of an IVF cycle, transfer technique may adversely affect implantation and pregnancy. Optimizing ET will allow providers to maximize successful interaction of embryo and endometrium, leading to the establishment of a viable pregnancy. These data suggest that the presence of blood during a routine, easy ET is not detrimental to live birth.

Authors' Note

The views expressed in this manuscript are those of the authors and do not reflect the official policy or position of the Department of the Army, Department of Defense, or the US Government.

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Declaration of Conflicting Interests

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