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Original Research Article

Does the presence of blood on the catheter or the degree of difficulty in embryo transfer affect the outcome in ART

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ABSTRACT

Background: Implantation may be impacted by the embryo transfer (ET) method. It's uncertain whether postprocedural blood at the transfer catheter tip is a true indicator of transfer difficulty because previous studies that examined its impact have produced conflicting findings. Our goal was to calculate the impact of blood at the moment of Embryo Transfer and the degree of difficulty associated with it on live birth rates (LBR).
Methods: This was a retrospective study conducted at Kamlesh Tandon test tube baby centre, Agra from July 2022 to April 2024. Patients underwent COS followed by Oocyte retrieval and fresh embryo transfers. Patients with high Serum Oestradiol levels and more than 15 oocytes were deferred for a fresh embryo transfer. A freeze-all policy was followed by a subsequent freeze thawed embryo transfer cycle. The Clinical Pregnancy Rates and the Live Birth Rates were calculated.
Results: Generalized estimating equations (GEE) for logistics regression with exchangeable correlation with robust variance was used to estimate the adjusted and unadjusted ORs in this retrospective cohort study. After conducting univariate modelling, all relevant confounders were taken into account in the final multivariate (adjusted) GEE model. At the moment of transfer, the ART specialist would subjectively assess embryo transfers as easy, medium or hard. Blood found at ET was linked to more challenging ETs, retained embryos in the catheter and mucus in the catheter. The degree of difficulty for ET had an adverse effect on the live birth rate (LBR), while ET with blood did not correlate with live birth in the univariate study. The only variables linked to an elevated LBR in the final multivariate GEE model, which took into account a patient's repeated cycles, were the blastocyst transfer, female age and the difficulty of the ET. The presence of blood in the transfer catheter was not linked to the chance of pregnancy and, therefore, was not an independent predictor of cycle outcome after correcting for confounding variables. This suggests that the transfer's inherent complexity and its difficulties are factors that have a substantial negative predictive impact on pregnancy outcomes.
Conclusions: 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.

Keywords: ART, Blood on the catheter, Difficult transfer, Degree of difficulty, Embryo transfer

INTRODUCTION

The procedure of embryo transfer (ET) is critical in establishing the optimal environment for a successful implantation. Research indicates that the following factors increase the chance of getting pregnant: using ultrasound guidance, bladder distension, mock transfers, embryo after loading, using soft-tip catheters and where the embryo deposition occurs inside the uterus.[1-9] Two additional significant factors are blood-contaminated catheter tips

and difficulties encountered during the embryo transfer process.[10] There has been debate over research on the presence of blood on the tip of the transfer catheter following Embryo Transfer. Some studies have found no effect, while several have reported a considerable decrease in the chance of a successful pregnancy.[11-16] It is usually considered that blood in the catheter during a transfer represents endometrial or cervical bleeding, which could potentially pose problems during implantation.[17,18] A traumatic transfer may be directly linked to the presence of blood and the difficulty of the transfer.

Difficult ETs are defined as those that are judged challenging, need more time or effort, stiffer catheters or special tools like a tenaculum. The current literature is divided on the influence of transfer difficulty in competent hands-on cycle success; some studies show no effect, while others show a markedly lower pregnancy rate.[4,19-25]

Considering the contradictory data about the relationship between blood on the catheter and the difficulty of ET with cycle outcome, we aimed to investigate the relationship between these variables and success, following in vitro fertilization (IVF). The impact of blood and the level of difficulty have not been documented while utilizing the ET afterload approach, despite the possibility that it will benefit pregnancy. This study aimed to quantify the impact of blood during embryo transfer and the challenge of embryo transfer on live birth following in vitro fertilization.

METHODS

This was a retrospective conducted at Kamlesh Tandon test tube baby centre, Agra from July 2022 to April 2024. Patients underwent COS followed by Oocyte retrieval and fresh embryo transfers. Patients with high serum oestradiol levels and more than 15 oocytes were deferred for a fresh embryo transfer.

A freeze-all policy was followed by a subsequent Freeze Thawed Embryo transfer cycle. The Clinical Pregnancy Rates and the Live Birth Rates were calculated. All patients were administrated GnRH-agonist long (luteal phase) protocols. Stimulation was done with either HMG or rFsh (Menotas HP or GONAL-F) and the dosage of Gonadotrophin was adjusted based on ovarian response and was assessed by transvaginal ultrasound and serum oestradiol level. If two or more follicles reached a mean diameter≥18 mm, the patients were injected with 10,000 IU of recombinant hCG (Ovitrelle). Ovum pick up was performed transvaginal, 34-38 hours after hCG injection.

Within an hour or so of oocyte retrieval, the oocytes were denuded and all matured oocytes were subjected to ICSI. All fertilized oocytes were cultured for five days in culture media (Single step media), with a shift in fresh media on day 3. To avoid the cancellation of embryo transfers, the clinic's policy was to only choose patients showing at least five high-quality embryos on day three to extend embryo culture until day five. Embryos on day three were classified based on the grading by Kamardi et al.[26]

Grade 1

Embryos were defined as 4-6 cells on day two, eight or more cells on day three, equal, fragmentation<10% and no multinucleated blastomeres.

Grade 2

Embryos were defined as 2-3 cells on day two, 6-7 cells on day three, equal or less equal, 10- 20% fragmentation and no multinucleated blastomeres Grade-3: Embryos were defined as 0-2 cells on day two, 1-5 cells on day three, unequal, fragmentation>25%, with or without multinucleated blastomeres.

Grade 1 and 2 embryos were considered top-quality embryos. Blastocysts were graded based on the Modified Grading by Richardson et al, which is defined by the size of the blastocele cavity (Early, Full, Expanded), the size of inner cell mass; and trophectoderm layer distribution.

Grade A and B blastocysts on day five were considered top-quality embryos.[27] Early blastocysts or blastocysts with no inner cell mass were included among Grade-C blastocysts. The above grading appeared to be simpler, easier and embryologist-friendly. There was no embryo transfer done if serum oestradiol values were more than 3000 pg/ml on the day of HCG Trigger and more than 15 oocytes were retrieved. These patients were treated as cases of OHSS and were treated accordingly.

ART technique for embryo transfer

Abdominal ultrasonography guidance was used during all ET procedures, cook embryo replacement catheters (Guardia Access Embryo Transfer Catheter KJETS-7019) were used. The afterload method was used to finish the embryo transfer process. 5 First, under ultrasound supervision, only the outer sheath of the double catheter was introduced into the cervix and progressed to just past the internal cervical os.

Following confirmation of the passage, the embryologist loaded the embryos into an inner catheter which was introduced into the outer sheath of the catheter in situ. After being inserted through the outer catheter, the inner catheter containing the embryos was gradually progressed under ultrasonic guidance until it was one centimetre deep from the fundal endometrium.

The embryos were then implanted. The inner catheter was then taken out and returned to the lab so that any blood or mucus on or in the transfer catheter could be examined under a microscope. The embryologist also checked to see if there was a retained embryo in the transfer catheter. While waiting for the embryologist's evaluation, the transferring physician kept the outside catheter in place

during this period. If no embryos were retained, the outside catheter was taken out. If a detained embryo was found, it was transferred again via the outer sheath and unloaded into an inner catheter. Before leaving the transferring physician graded the difficulty of the ET as easy, medium or hard before being told if there was microscopic blood or mucous on the catheter. A transfer that is described as "easy" is completed without any difficulties or extra equipment.

A "hard" transfer is one in which finishing the catheter's passage into the endometrium requires more time or approaches (such as using a rigid transfer catheter or tenaculum). If a transfer does not fit the requirements for an easy or hard transfer, it is labelled as "medium." During this time, the difficulty grade was the same for all transferring physicians. If a fellow carried out the transfer, the supervising attending and the fellow discussed and decided on the difficulty grade. When a fresh embryo transfer was anticipated for a patient, luteal phase support was administered as vaginal progesterone pessary 400 mg twice a day, injectable progesterone 100 mg SC daily and Tab Duphaston 10 mg daily.

Day 11 of the embryo transfer process was the day for B HCG testing. After 11 days of embryo transfer, serum BHCG levels greater than 100 Miu/ml were regarded as positive for pregnancy. In these instances, luteal phase support persisted until a transvaginal ultrasound was used to do a clinical pregnancy evaluation. Starting at 12 weeks, the luteal phase assistance was progressively reduced over two weeks. All of the embryos in the women who received frozen embryo transfers were vitrified. On the 22nd day of the previous cycle, the women in the scheduled cycle of the FET cycle received a GnRH agonist depot dose of 3.6 mg SC and 10 mg was administered to induce menstruation by administering 10 mg of Tab.

Progesterone twice a day for ten days or by starting a combination of OCP on the first day of menstruation of the preceding cycle. Baseline hormone tests were performed, estrogen tablets were prescribed for endometrial growth and patients were started on hormone replacement therapy (HRT) with gradual dose increases. Ultrasonography was performed starting on day 9 to measure endometrial thickness, vaginal progesterone pessary 400 mg twice a day, injectable progesterone 100 mg SC daily and tab Duphaston 10 mg daily were started as soon as the endometrial thickness crossed 7 mm with good triple line as luteal phase support, till the day of testing of serum B HCG. Up to 2 or 3 thawed Blastocysts were transferred. Details about the outcome of the pregnancy were obtained using a review of obstetrical medical records and neonatal medical records.

Statistical analysis

Generalized estimating equations (GEE) for logistics regression with exchangeable correlation with robust variance was used to estimate the adjusted and unadjusted ORs. For parametric distributions, the data were evaluated using mean±standard deviation (SD) and for nonparametric distributions, median with ranges. Generalized estimating equations (GEEs) modelling with nesting for repeated cycles within a patient was used for the major data analyses. Using the GEE models, continuous and dichotomous variables were evaluated according to the kind of data distribution. To look for parameters related to blood presence at ET and factors related to live birth, unadjusted univariate GEE models were used.

Adjusted multivariate models then contained variables that were determined to be statistically significant in unadjusted GEE modelling. Modified GEE models take into account nesting for multiple cycles within a patient as well as variables like age, blastocyst transfer difficulty and age, to find additional factors linked to live birth, the blastocyst transfer difficulties, the quantity of embryos transferred, the blood on the transfer catheter and the interactions between these variables were used. Based on the presence or absence of blood and the complexity of the transfer, the results of IVF cycles and live births were compared using the w2. P values less than 0.05. were regarded as significant statistically. The statistical analysis was carried out with IBM, Armonk, New York's SPSS program (version 22).

RESULTS

In this study, 142 patients undergoing 153 ET cycles were included. The mean+SD patient’s age was 34+5 years. There were 135 (88.23%) transfers without blood in the catheter and 18 (11.76%) 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. There were 138 easy transfers, 9 medium difficulty transfers and 6 difficult transfers as recorded by the transferring physician. Easy transfer had a clinical pregnancy rate and live birth rate of 52.89% and 89.04% respectively.

Medium easy transfer had a clinical pregnancy rate and live birth rate of 66.66% and 83.33% respectively, while difficult transfer had the least clinical pregnancy rate and live birth rate of 50.00% and 66,66%. The clinical pregnancy rate and live birth rate in group with no blood on catheter was 52.59% and 88.73% respectively, while in the group with blood on catheter was 66.66% and 83.33% respectively. When controlling for these factors in the model, the presence of blood was not significantly associated with cycle outcomes.

The clinical pregnancy rates, spontaneous abortion rates, ectopic pregnancy rates and live birth rates in the no blood at ET and Blood at ET were 52.59%, 9.95%, 1.40% and 46.66% and 66.66%, 8.33%, 8.33% and 55.55%. Although the live birth rates were better in the blood on ETgroup.it was not statistically significant. The data was further split into fresh and frozen ET subgroups for comparison.

Patient's age, the thickness of the endometrium and the endometrial grade were not associated with blood at ET. The outcome when compared with respect to live birth rate, clinical pregnancy rate, spontaneous abortion rate and ectopic pregnancy rate had no statistical significance as none of them had a p value of <0.05. Transfers without the presence of blood in the catheter had an 46.66% live birth rate versus 55.55% for transfers with blood. In univariate GEE models, variables associated with live birth included the patient's age, parity, blood at the time of ET and peak endometrial thickness. 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, p values were not statistically significant, variables considered here were age, parity, peak endometrial thickness and blood at transfer.

Table 1: Baseline and cycle stimulation comparison between two groups (n=153) (135+18).

Outcome	No blood at ET	Blood at ET
Presence of mucous on catheter	0	0
Retained embryos	0	0
Age in years	35	37
Endometrial thickness in mm	10	9.3
Fresh embryo transfer	104	17
Frozen embryo transfer	31	1

Table 2: Break up of Pregnancy and live birth rate based on degree of transfer difficulty (n=153).

Variable	Number		Clinical pregnancy		Live birth	
	Fresh	**Frozen**	**Fresh**	**Frozen**	**Fresh**	**Frozen**
Easy transfer	111	27	56	17	50	15
Medium transfer	4	5	2	4	2	3
Difficult transfer	6	0	3	0	2	0
Total	121	32	61	21	54	18
Final Total	153		82		72	

Table 3: Pregnancy and live birth based on degree of transfer difficulty (n=153).

Variable	Number	Clinical pregnancy		Live birth	
Easy transfer	138	73	52.89%	65	89.04%
Medium transfer	9	06	66.66%	05	83.33%
Difficult transfer	6	03	50.00%	02	66.66%
Total	153	82		72	

Table 4: Comparisons of pregnancy outcome in patients with and without blood on the embryo transfer catheter (n=153).

Outcome	No blood at ET	%	Blood at ET	%
Number	135		18	
Clinical pregnancy	71	52.59	12	66.66
Spontaneous abortion	7	9.85	1	8.33
Ectopic pregnancy	1	1.4	1	8.33
Live birth	63	46.66	10	55,55

Table 5: Comparisons of pregnancy outcome in patients with and without blood on the catheter embryo transfer with fresh and frozen embryo transfer.

Outcome	No blood at ET				Blood at ET			
	Fresh		**Frozen**		**Fresh**		**Frozen**	
Number	104	+	31	=135	17	+	01	=18
Clinical pregnancy	51	+	20	=71	11	+	01	=12
Spontaneous abortion	04	+	03	= 07	01	+	00	=01
Ectopic pregnancy	01	+	00	=01	01	+	00	=01
Live birth	46	+	17	=63	09	+	01	=10

Table 6: Pregnancy and live birth rates comparisons in patients with and without blood on the catheter at embryo transfer.

Outcome	No blood at ET	Blood at ET	P value
Live birth rate	88.73	83.33	0.393
Clinical pregnancy rate	52.59	66.66	0.626
Spontaneous abortion	9.85	8.33	0.754
Ectopic pregnancy	1.4	8.33	0.79

Table 7: 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	0.09	Ref	2.39 (0.88–6.48)	0.07	Ref	1.63 (0.92–7.52)
Age	0.94		0.99 (0.94–1.05)	0.9		1.01 (0.95–1.06)
Peak endometrial thickness	0.32		1.08 (0.93–1.25)	0.34		1.09 (0.92–1.29)
Parity	0.14	Ref	0.59 (0.29–1.19)	0.07	Ref	1.63 (0.92–7.52)

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 and a significant negative effect of transfer difficulty, as well as a recent meta-analysis demonstrating similar results for both blood and transfer difficulty.[17,18,20,28] 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] Our study highlights that use of various modalities like use of USG guidance, stabilisation of the cervix straightening of cervical canal, previous cervical dilatation before a suspected difficult

Embryo transfer, would the key factors to convert difficult embryo transfer to medium difficult and make the outcome similar or even little better than the simple transfer. Quite often the simple appearing transfer becomes difficult ones which is realised after the procedure is over. Hence it is advisable to keep all the modalities handy and easily available at the time of embryo transfer. 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.[13] However, the authors did not assess for correlation between transfer difficulty and the presence of 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. Considering the studies comprising day 3 to day 5 transfer and the above parameters, the most significant variable would be the implantation potential for a cleavage stage embryo to a blastocyst stage embryo. Hence, in our study we have done only blastocyst (Day 5) transfers. Given the increasing number of blastocyst transfers being done now 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. 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. It is to be noted that using direct insertion suggested a negative correlation with blood on catheter.[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 modelling 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 USG as well as hystero-laparoscopy 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 although, the ETs were performed by the same ART consultant and attending providers, which may be a limitation but could also make the study more generalizable.

CONCLUSION

Embryo transfer 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.

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