Contamination of Embryo Transfer Catheter and Treatment Outcome in In Vitro Fertilization

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Purpose: The effect of embryo transfer (ET) catheter contamination with mucus and/or blood on treatment outcome in an in vitro fertilization (IVF) program was evaluated.

Methods: One thousand four ET procedures in 877 patients having conventional IVF in the long (1189 cycle) and short (15 cycles) protocol were analyzed to determine the impact of catheter contamination on the incidence of retained embryos and the clinical pregnancy rate.

Results: Catheter contamination with mucus and/or blood is a feature of difficult ET. Embryos were significantly more likely to be retained when the transfer catheter was contaminated with mucus (17.8 versus 3.3%) or blood (12 versus 3.3%). When the catheter was contaminated and an embryo(s) was retained and immediately retransferred, the pregnancy rate was not compromised. The pregnancy rate was significantly reduced when the ET catheter was contaminated with blood (15.5 versus 27.1%; $P = 0.002$), but no embryo was retained in the catheter set.

Conclusions: Catheter contamination compromises the treatment outcome in IVF only when there is no associated retained embryo(s). As increased vigilance in searching for extruded embryos may not be practical, we suggest that cervical mucus should be routinely aspirated and ET performed as atraumatically as possible.

KEY WORDS: difficult embryo transfer; catheter contamination; residual embryos; pregnancy rate.

INTRODUCTION

Despite the advances made in the past few years in superovulation regimens and in vitro fertilization (IVF) and embryo culture techniques, implantation rates fol-

lowing embryo transfer (ET) have remained at 12–15% (1). While attention is rightly being focused on understanding the mechanism involved in implantation and ways of improving it, workers in the field are also aware that ET techniques influence the pregnancy rate. The inability to place embryos in the uterine cavity and factors preventing them from remaining there adversely affect the pregnancy rate. Catheter contamination with mucus and/or blood has been associated with an increased embryo expulsion rate, with a concomitant decrease in pregnancy rate (2). In a recent review of 1204 ET procedures (3) we reported that although catheter contamination was associated with an increase in embryo retention rate, the pregnancy rate was not compromised provided that the expelled or retained embryos were discovered and immediately retransferred. However, whether catheter contamination on its own, especially when no embryos are retained in the catheter, compromises the treatment outcome remains to be determined. In this study we have reanalyzed our data to test the null hypothesis that the pregnancy rate following ET is not compromised in cases in which no residual embryos are found but the ET catheter is contaminated with mucus and/or blood.

MATERIALS AND METHODS

Between January 1991 and December 1995 data about 1204 ET procedures in 877 patients having IVF with the long (1189 cycles) and short (15 cycles) buserelin protocol at Midland Fertility Services were analyzed retrospectively.

The methods of ovulation induction and monitoring in both the long and the short protocol using buserelin acetate (Suprefact; Hoechst, Hounslow, UK) and human menopausal gonadotrophin hMG; Personal; Serono Laboratories, Welwyn Garden City, UK; or

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Humegon; Organon, Cambridge, UK) have been described previously (3). Oocyte collection was performed under i.v. sedation with pethidine and midazolam 35 hr after giving 10,000 IU human chorionic gonadotrophin (hCG; Profasi; Serono) i.m. to induce oocyte maturity.

Semen preparation, oocyte insemination, and embryo culture were performed using standard procedures. Evidence of fertilization was established by light microscopy 16 to 18 hr after insemination.

Two days after oocyte collection, a maximum of three embryos in 20 μ l of culture medium was loaded into a transfer catheter with air bubbles before and after. Patients with an anteverted uterus attended for ET with their bladder full whereas those with axial or retroverted uterus attended with an empty bladder. All ETs took place with patients in the dorsal position. The cervix was exposed with a bivalve speculum and cleaned with a dry sterile swab or a swab soaked in phosphate-buffered saline, without aspirating the cervical mucus prior to ET. The loaded catheter was gently passed through the cervix to a distance of 0.5–1 cm from the fundus. When difficulty was encountered with passing the catheter, a single-toothed vulsellum (Holborn, Kent, UK) was used to pull on the anterior lip of the cervix to straighten the cervical canal. If this was unsuccessful, a metal catheter (Bristol Introducer; Casmed, Cheam, Surrey, UK) with an introducer was then used.

After deposition of the embryos in the uterine cavity, the catheter was withdrawn and passed to the embryologist while maintaining pressure on the syringe plunger. Under the microscope, the catheter was checked for embryos retained within its lumen or adherent to mucus and/or blood contaminating it. This was performed by flushing the catheter with culture medium in the well of a Nunc four-well multidish (Nunc A/S, Kamstrup, Denmark). When embryos were found, they were reloaded and immediately retransferred with the same catheter, after it had been rinsed with culture medium. The ease of transfer and the state of the catheter were recorded by the embryologist. Catheters were recorded as clean or contaminated with mucus, blood and mucus, or blood. For the purpose of this study catheters recorded as stained with mucus and blood were grouped together with those stained with blood only.

A pregnancy test was performed 2 weeks after ET, and if it was positive, patients had an ultrasound scan 2 weeks later to establish the number of gestational sacs and embryonic viability and to exclude ectopic pregnancy. Clinical pregnancy is defined as the pres-

ence of an intrauterine gestation sac with yolk sac, fetal pole, and fetal heart pulsations.

Of the 1204 ET procedures, the state of the catheter was not recorded in 3 cases, and in 1 case although the patient became pregnant she was lost to follow-up before ultrasound scan. For the purpose of this study, patients were divided into three groups depending on whether the catheter after ET was clean [Group 1; $n = 920$ (76.4%)], contaminated with mucus [Group 2; $n = 90$ (7.5%)], or contaminated with blood with or without mucus [Group 3; $n = 191$ (15.9%)]. For statistical analysis, independent t test, Fisher's exact test, and chi-square test were used as appropriate. Statistical significance was set at $P < 0.05$.

RESULTS

Excluding the three transfer procedures in which the state of the catheter and the ease of transfer were not recorded, contamination of the transfer catheter with mucus and blood occurred significantly more often when the transfer procedure was difficult. Difficult ET was significantly more likely to be associated with a bloody than with a mucus-stained catheter (Table I).

Of the 1201 ETs analyzed, embryos were retained in 69 (5.7%) cases necessitating multiple attempts at ET. Embryos were significantly more likely to be retained when the catheter was contaminated with mucus and blood than when it was clean. There was no significant difference in the rate of embryo retention when catheters were stained with either mucus and/or blood (Table I). All embryos were successfully transferred by the third attempt.

Thirteen operators performed ET procedures during the study period (3 doctors and 10 nurses). Embryos were no more likely to be retained when nurses performed the procedure (6%) than when doctors were involved (4.4%). When the catheter was contaminated, no differences occurred in the clinical pregnancy rate among the various operators who performed ET during the study period.

There were no significant differences with regard to demographic variables, the response to ovulation induction, the number of embryos transferred, and the clinical pregnancy rate between those patients who had all their embryos transferred at the first attempt and those requiring multiple attempts (Table II).

The clinical pregnancy rate was significantly lower when the transfer procedure was difficult [58/305 (19%) versus 238/895 (26.6%); $P = 0.008$]. However,

Table I. Catheter Contamination in Relation to Difficulty Encountered at ET, Retained Embryos, and Clinical Pregnancy Rate

	Clean (Group 1) (n = 920)	Mucus (Group 2) (n = 90)	Blood (Group 3) (n = 191)
Embryos retained	30 (3.3%)*	16 (17.8%)*	23 (12%)*
Difficult transfer	122 (13.3%)*	49 (54.4%)*	134 (70.2%)*
Ectopic pregnancy	16 (6.1%)	0	0
Clinical pregnancy			
Overall	247 (26.9%)*	18 (20.0%)	31 (16.2%)*
Easy transfer	218/797 (27.4%)	11/41 (26.8%)	9/57 (15.8%)
Difficult transfer	29/122 (23.8%)	7/49 (14.3%)	22/134 (16.4%)
No embryo retained	241/889 (27.1%)*	13/74 (17.6%)*	26/168 (15.5%)*
Embryos retained	6/30 (20.0%)	5/16 (31.3%)	5/23 (21.7%)

* $P = 0.07$.** $P = 0.01$.*** $P = 0.002$.**** $P = 0.000001$.

there was no significant difference in pregnancy rate when the ease of ET was analyzed in relation to the state of the ET catheter (Table I).

The overall clinical pregnancy rate was significantly reduced when the ET catheter was contaminated with blood than when it was clean. This was not the case when embryos were retained, found, and immediately retransferred into the uterus. However, when catheter contamination was not associated with retained embryos, the pregnancy rate was significantly reduced when the ET catheter was stained with mucus and/or blood (Table I). The embryo retention rate with a Wallace (Smiths Medical Distribution Ltd., London) catheter [10/173 (2.3%)], although lower, was not

significantly different from that for the Embryon catheter (Rocket of London, Watford, UK) [58/928 (6.3%)] and other less commonly used ET catheters [7/98 (7.1%)].

DISCUSSION

Clinical studies have shown that good ET technique improves the chance of conception following IVF (4–6). While factors such as the choice of ET catheter, position of the patients, and use of tocolytics or general anesthesia (7) have not been shown to influence the treatment outcome, the technical ease or difficulty of the procedure (8), possibility of damage during transfer (9), and contamination of the ET catheter tip with cervical flora (10) mucus and/or blood (2,4,5) may be important.

Contamination of the ET catheter with mucus and/or blood has been associated with expulsion or retention of embryos in the transfer set (2,3). There may be several reasons for this: mucus and/or blood may cause mechanical blockage of the catheter opening; the contaminants may cause embryos to adhere to the catheter when it is withdrawn; and embryos may adhere to the contaminant on the sides of the catheter and be dragged to the lower pole of the uterus, the cervical canal, and even outside the uterus and be lost in the vagina upon withdrawing the catheter. We have shown that although catheter contamination is associated with an increased rate of retained embryos, the pregnancy rate is not compromised when the embryos are found at inspection of the catheter and immediately retransferred (3).

The decrease in pregnancy rate when the ET catheter was contaminated with blood was not caused by

Table II. Demographic Data, Response to Ovulation, Number and Grade of Embryos Transferred, and Clinical Pregnancy Rate in Patients with and Without Retained Embryos at Transfer

	Transfer procedures	
	With embryos retained (n = 69) ^a	Without embryos retained (n = 1135)
Patient's age (years) ^b	34.1 (4.6)	33.9 (4.6)
Duration of infertility (years) ^b	5.8 (3.1)	5.8 (3.4)
Baseline FSH ^c (IU/L) ^b	7.7 (2.6)	7.8 (2.9)
Total dose of hMG (ampoules) ^b	28.1 (11.3)	29.2 (11.1)
Number of follicles aspirated ^b	15.7 (10.0)	14.0 (8.2)
Number of oocytes collected ^b	11.2 (6.3)	10.2 (6.3)
Number of oocytes fertilized ^b	6.3 (4.0)	5.9 (4.1)
Number of embryos transferred ^b	2.8 (0.5)	2.6 (0.6)
Cumulative embryo score ^b	16.3 (9.0)	16.0 (6.9)
Clinical pregnancy rate ^b	16/69 (23.2%)	280/1135 (24.7%)

^a n = number of ET procedures.^b Data are the mean (SD).^c Follicle stimulating hormone.

retained or expelled embryos. This is similar to the findings of Wisanto *et al.* (5), who reported a 7.7% pregnancy rate when cervical bleeding occurred compared with 21.3% when no cervical bleeding was observed. They found no significant difference in pregnancy rate when easy transfer was compared with transfers requiring cervical dilatation.

Why the clinical pregnancy rate should be compromised when a blood-stained catheter is not associated with retained embryos is conjectural. Embryo transfer is a blind procedure and deductions have to be made from indirect observations. However, it seems that failure of the proper placement of embryos at the time of transfer may occur frequently. Given that extruded or residual embryos have been found in places other than in or attached to the transfer catheter (11), evaluation of the transfer set alone may result in a significant underestimation of the embryo loss rate. When the transfer catheter is contaminated but no retained embryos are found, not all the transferred embryos may be within the uterine cavity. Expelled embryos have been found on the vaginal speculum, in the cervix, and in the strand of mucus adhering to the catheter tip (at withdrawal) as well as in the catheter itself (11). Whether this would explain the significant reduction in pregnancy rate when the ET catheter was contaminated with no associated extruded embryos needs to be confirmed in a prospective study.

The only method of confirmation that embryos have been successfully deposited within the uterine cavity is for patients to have an intrauterine pregnancy. A single intrauterine pregnancy when multiple embryos have been transferred is no guarantee that the other embryos were correctly deposited in the uterine cavity. Ensuring that embryos are placed in the uterine cavity without evoking bleeding or allowing catheter contamination with mucus would increase the likelihood that transferred embryos remain in the uterus.

In summary, we reject the null hypothesis and conclude that the pregnancy rate following ET is compromised in cases in which no residual embryos are found but the ET catheter is contaminated with mucus and/or blood. When catheter contamination is associated with residual embryos, the pregnancy rate is not compromised provided the embryos are found and immediately retransferred. Because increased vigilance in

searching for extruded embryos may not be practical in a busy unit, we consider it prudent to aspirate cervical mucus and perform the ET as atraumatically as possible.

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