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Multiple attempts at embryo transfer: does this affect in-vitro fertilization treatment outcome?

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In this study, we retrospectively analysed data from 877 patients who had 1204 embryo transfer procedures following in-vitro fertilization (IVF) at Midland Fertility Services, UK, between January 1991 and December 1995 to investigate the factors contributing to failure of embryo transfer at first attempt and the impact of immediate retransfer of retained embryos on the treatment outcome. Embryos were significantly more likely to be retained when the embryo transfer catheter was contaminated with mucus (3.3 versus 17.8%, $P = 0.000001$) or blood (3.3 versus 12%, $P = 0.00001$) and when the transfer procedure was difficult compared with when it was easy (20.3 versus 0.8%, $P = 0.00001$). There was no significant difference in the clinical pregnancy rate between those who had all their embryos transferred at the first attempt (24.7%) and those who required more than one attempt (23.2%). The types of embryo transfer catheter used in the unit did not show any difference in terms of embryo retention. Although we recommend aspiration of cervical mucus in order to reduce the rate of retained embryos, there is no evidence from our study to suggest that pregnancy rate is compromised when embryos are retained, provided they are discovered and immediately retransferred into the uterine cavity. Immediate retransfer is more convenient to the patients and reduces the laboratory workload without compromising the treatment outcome.

Key words: IVF/pregnancy rate/retained embryos

Introduction

The placement of multiple embryos in the mid-fundal area of the uterus is crucial to obtaining an optimal pregnancy rate in in-vitro fertilization (IVF) (Rosenlund *et al.*, 1996). With good quality embryos, optimal pregnancy rates may still not be achieved if the transfer technique is defective. Unfortunately, the technique of embryo transfer is essentially blind (Mansour *et al.*, 1994) and there is no guarantee that the embryos will remain in the uterine cavity after the procedure. Embryos have been found retained in catheters (Visser *et al.*, 1993), on the cervix and on the vaginal speculum (Poindexter *et al.*, 1986), whilst Mansour *et al.* (1994) showed that expulsions are more likely when cervical mucus is not aspirated prior to transfer. One study (Visser *et al.*, 1993) noted a significant reduction in pregnancy rate following retention of embryos in the transfer set, suggesting that the act of retransfer may compromise the retransferred embryos or those retained in the uterus. As pregnancy rates following embryo replacement are influenced by several factors, it seems that simple reasons are possibly being used to explain a complex phenomenon. In this study, we investigated the factors contributing to failure of embryo transfer at first attempt and our practice of immediate retransfer of retained embryos without changing the transfer catheter.

Materials and methods

We retrospectively analysed data on 877 patients who had 1204 embryo transfer procedures following IVF using the long (1189 cycles) and short (15 cycles) protocols at the Midland Fertility Services (Aldridge, UK) between January 1991 and December 1995.

Ovulation induction in the long protocol consisted of down-regulation with buserelin acetate (Suprefact; Hoechst, Hounslow, UK) starting in the mid-luteal phase for 3 weeks, following which human menopausal gonadotrophin (HMG) (Pergonal; Serono Laboratories, Welwyn Garden City, UK, or Humegon; Organon, Cambridge, UK), two to six ampoules per day, was added. Patients who had the short protocol commenced buserelin acetate on the first day of their menstrual cycle with HMG added from about the third day. Monitoring was carried out by transvaginal scan and oestradiol estimation on day 9 of HMG stimulation. Buserelin acetate and HMG were stopped, and i.m. human chorionic gonadotrophin (HCG) 10 000 IU (Profasi; Serono) were given when it was estimated that patients would have two or more follicles >17 mm diameter. Oocyte collection was scheduled 35 h after HCG injection by transvaginal ultrasound-guided follicle aspiration. The procedure was performed under i.v. sedation with pethidine and midazolam.

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

A maximum of three embryos was loaded in the catheter 2 days following oocyte collection, in 20 µl of culture medium using air bubbles before and after. If the patient had an anteverted uterus she was asked to attend for embryo transfer with a full bladder to straighten the cervical canal. With the patient in the dorsal position, a bivalve speculum was placed in the vagina to visualize the cervix. The cervix was cleaned with a dry sterile swab or swab soaked in phosphate buffered saline without aspirating cervical mucus prior to embryo transfer. The loaded catheter was gently passed through the cervix to a distance of 0.5–1 cm from the fundus. If there was any difficulty in threading the catheter through the cervix, a single-toothed vulsellum (Holborn, Kent, UK) was used, and eventually a metal catheter (Bristol Introducer; Casmed, Cheam, Surrey, UK) if required.

After deposition of embryos in the uterine cavity, the catheter was withdrawn and passed to the embryologist whilst maintaining the pressure on the syringe plunger. Under the microscope, the catheter was checked for embryos retained within the lumen or adherent to

Table I. Demographic data, response to ovulation induction, the number and grade of embryos transferred in patients with (group 1) and without (group 2) retained embryos at transfer

	Group 1 (n = 1135)	Group 2 (n = 69)
Patient's age (years)	33.9 (4.6)	34.1 (4.6)
Partner's age (years)	35.9 (6.2)	36.2 (6.1)
Duration of infertility (years)	5.8 (3.4)	5.8 (3.1)
Baseline FSH (IU/l)	7.8 (2.9)	7.7 (2.6)
Baseline LH (IU/l)	5.1 (3.1)	4.7 (1.8)
Total dose of HMG (ampoules)	29.2 (11.1)	28.1 (11.3)
Number of follicles aspirated	14 (8.2)	15.7 (10.0)
Number of oocytes collected	10.2 (6.3)	11.2 (6.3)
Number of oocytes fertilized	5.9 (4.1)	6.3 (4.0)
Number of embryos transferred	2.6 (0.6)	2.8 (0.5)
Cumulative embryo score	16.0 (6.9)	16.3 (9.0)

Values are mean (SD).
n = number of embryo transfer procedures.
FSH = follicle stimulating hormone, LH = luteinizing hormone, HMG = human menopausal gonadotrophin.

mucus and/or blood contaminating it. This was performed by flushing the catheter with culture medium in the empty well of a Nunc four-well multidish (Nunc A/S, Kamstrup, Denmark). If embryos were found, they were re-loaded and immediately retransferred with the same catheter after being cleaned with culture medium. The ease of transfer and the state of the catheter were recorded by the embryologist on the embryo transfer form. 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 following embryo transfer, and if positive, patients were booked for an ultrasound scan 2 weeks later to establish number of gestational sacs and embryonic viability, and to exclude ectopic pregnancy.

For the purpose of this study, patients were divided into two groups depending on whether their embryos were all successfully transferred at the first attempt [group 1, n =1135 (94.3%)] or whether multiple attempts were required to transfer all the embryos [group 2, n = 69 (5.7%)]. Categorical data were summarized by proportions. Pearson's χ^2 and Fisher's exact tests were used to compare proportions between discrete variables. Odds ratio estimates (OR) and 95% confidence intervals (CI) for the OR were calculated to compare the odds of having embryos retained when the embryo transfer procedure was difficult and when the transfer catheter was contaminated. Non-categorical data were compared using t-test. Statistical significance was set at $P < 0.05$.

Results

There were no significant differences between the two groups in the demographic variables, the response to ovulation induction, the number and the cumulative embryo score (Steer *et al.*, 1992) of the embryos transferred (Table I).

Of the 1204 embryo transfer procedures, the state of the catheter was not recorded in three cases and in one case, although the patient became pregnant she was lost to follow-up before ultrasound scan.

Compared to when the catheter was clean following the initial transfer, embryos were significantly more likely to be retained when the catheter was contaminated with mucus (3.3 versus 17.8%, $P = 0.000001$, OR = 6.4, 95% Cl = 3.3–12.3) and blood (3.3 versus 12%, P = 0.000001, OR = 4.1, C1 = 2.3–7.1). There was no difference between the rate of embryo retention when the catheter was stained with mucus (17.8%) or blood (12%).

Excluding the three transfer procedures in which the ease of transfer was not recorded, embryos were significantly more likely to be retained when the transfer procedure was difficult compared to when it was easy (20.3 versus 0.8%, $P = 0.000001$, OR = 32.4, CI = 14.6–71.7).

There was no significant difference in the clinical pregnancy rate in group 1 (24.7%) compared with group 2 (23.2%). In group 2, in 61 (88.4%) embryo transfer procedures, all embryos were successfully replaced at the second attempt, whilst a further eight (11.6%) required a third attempt to replace all the embryos. Interestingly, the clinical pregnancy rate remained relatively constant in the first (24.7%), second (23%) and third (25%) transfer attempts.

Of the 280 clinical pregnancies in group 1, there were 185 (66.1%) singletons, 81 twins (28.9%), and 14 triplets (5%), while in group 2 in 16 pregnancies, there were 13 singletons (81.3%), two sets of twins (12.5%) and one set of triplets (6.3%). This difference was not statistically significant. In two (13.3%) out of 15 cases where all three embryos were retained, multiple pregnancies were achieved (one twin and one triplet).

There were 16 (1.4%) ectopic pregnancies in this series, all of which occurred in patients in group 1. The types of embryo transfer catheter used in the unit did not show any difference in terms of embryo retention. The embryo retention rate with Wallace (Smiths Medical Distribution Ltd, London, UK) catheter [10/173 (2.3%)], although lower, was not significantly different compared with Embryon catheter (Rocket of London, Watford, UK) [58/928 (6.3%)] and other less commonly used catheters 7/98 (7.1%).

Discussion

Many factors have been implicated in the discrepancy between high fertilization and cleavage rates and the relatively low implantation rate. These include asynchrony between the stage of endometrial development and that of the embryos transferred

into the uterine cavity (Lewin *et al.*, 1989), low endometrial receptivity (Garcia, 1986), uterine contractions resulting from cervical stimulation (Craft *et al.*, 1981), leakage of culture medium through the cervix (Mansour *et al.*, 1994), the volume of transfer medium (Meldrum *et al.*, 1986), the type of embryo transfer catheter (Wisanto *et al.*, 1989), the technical ease or difficulty of embryo transfer procedures (Mansour *et al.*, 1994), contamination of the embryo transfer catheter tip with normal cervical flora at the time of embryo transfer procedure (Egbase *et al.*, 1996) and retention of embryos in the embryo transfer catheter (Garcia, 1986). To reduce the incidence of residual embryos, Poindexter *et al.* (1986) suggested that the catheter and syringe should be filled with medium rather than air behind the embryos, as this will prevent the compression of air when the end hole of the catheter is plugged with mucus. Although the practice in our unit is to fill the catheter and syringe with air behind the embryos, our results show no increase in the incidence of residual embryos. Our study is in agreement with the findings by various authors (Craft *et al.*, 1981; Wisanto *et al.*, 1989; Visser *et al.*, 1993) that catheter contamination by blood and/or mucus is associated with increased failure rate of the first attempt at embryo transfer.

In our study, all embryos were successfully transferred at the first attempt in 94.3% of cases. This is similar to the 83.4 and 95% successful first attempt rates reported by Wood *et al.* (1985) and Visser *et al.* (1993) respectively. The main reason for failed first attempt at embryo transfer was catheter contamination with mucus, blood or both. Most authors agree that blood and mucus contamination should be avoided whenever possible and one study, that of Mansour *et al.* (1994), advocated complete aspiration of cervical mucus in order to reduce the embryo expulsion rate. In their experiment they found expulsion of methylene blue dye in 57% of their cases when cervical mucus was not aspirated compared to 23% when mucus was aspirated. Although we would recommend aspiration of cervical mucus in order to reduce further the rate of retained embryos, findings from our study seem to suggest that the pregnancy rate is not compromised when embryos are retained, provided they are discovered and immediately retransferred into the uterine cavity.

The clinical pregnancy rate obtained in our study was not influenced by the number of attempts required to replace the embryos. This is in agreement with the findings by Wood *et al.* (1985) and Egan *et al.* (1990), who found no reduction in pregnancy rate in their failed first attempt cases. Visser *et al.* (1993) in their study reported a significant reduction (20.3 versus 3%) in pregnancy rate in cases of failed first attempt due to retention of embryos in the catheter. To overcome this problem, they suggested that retained embryos should be retransferred a day later. In fact, Poindexter *et al.* (1986) showed no pregnancy following immediate retransfer of expelled embryos but achieved 13.3% pregnancy rate when embryos were retransferred the following day. This contradicts our findings of no reduction in pregnancy rate following immediate retransfer.

One of the concerns about immediate retransfer of residual embryos is that the volume of fluid within the uterus may be at maximal capacity, risking 'flushing out' existing embryos either out of the cervix, or worse, into the Fallopian tubes (Poindexter *et al.*, 1986; Diedrich *et al.*, 1989). However, our study showed no ectopic pregnancies in the group of patients who required more than one attempt to complete the transfer of embryos into the uterine cavity. The rate of twin and triplet pregnancy was also not statistically different from those who had a successful first attempt at embryo transfer. In fact, one set of twins and one of triplets had the three embryos retained in the embryo transfer catheter and they were successfully retransferred on the second attempt.

In conclusion, retention of embryos in the embryo transfer catheter is related to contamination of the catheter with blood, mucus or both, and the difficulty of the embryo transfer procedures. The technique of catheter loading with air does not appear to affect the residual embryo rate, neither is the treatment outcome compromised by immediate retransfer of residual embryos. Immediate retransfer is more convenient to the patients and reduces the laboratory workload without compromising the treatment outcome.

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