Transfer technique and catheter choice influence the incidence of transcervical embryo expulsion and the outcome of IVF

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Introduction

Embryo transfer is the last decisive step on the way to a pregnancy in in-vitro fertilization (IVF). Whereas ovarian stimulation, oocyte retrieval, IVF and embryo culture are performed under visual control and can be adjusted correspondingly (Jones et al., 1982; Johnston, 1985; Diedtrich et al., 1987, 1988; Gembruch et al., 1988), the subsequent development of the embryo is beyond external control. Hence it is difficult to consider the influences on a potential initiated pregnancy at the time when the embryo is transferred.

Increasing implantation rates after embryo replacement attract much attention. Classically, crucial variables such as age, number and quality of transferred embryos have been directly linked to successful implantation. Very often, a single variable is evaluated alone in relation to a successful transfer. In this study, we aim to examine other factors related to the conditions of embryo transfer and evaluate their impact on pregnancy rates.

Materials and methods

Patients

Over a period of 15 months (Nov. 1996–Jan. 1998), 320 patients were included in this study. They were randomized on an alternate basis into two groups for embryo transfer either with an Edwards–Wallace catheter or with an Erlangen metal catheter. Randomization was also attempted within the same groups for 48 h and 72 h post-insemination transfer. The mean age of the female partners in the Edwards–Wallace group was 31.9 ± 4 years compared with 31.2 ± 2.9 years in the Erlangen group, while the duration of infertility was 5 ± 1.5 years and 4.7 ± 1.2 years respectively. Fifty patients randomly selected from each group were subjected to speculum examination 15 min following embryo transfer, during which any fluid leaking from the cervix was examined for the presence of embryos. In five patients a transvaginal–transmyometrial transfer was performed. The pregnancy rate appeared to be slightly higher in patients who had their embryos transferred at 72 h than in those patients who had their transfers at 48 h, but this difference was not significant in either group. (The ease with which the Erlangen catheter was used compared with that of the Wallace catheter was reflected in a significantly lower incidence of uterine sounding of cervical dilatation and bleeding.) Also there was a significant increase ($P = 0.0001$) in the mucus attached to the tip of the Wallace catheter and the embryos trapped compared with those of the Erlangen group ($P = 0.0007$). The pregnancy rate per embryo transfer was apparently higher in the Erlangen group than in the Wallace group but this difference was not significant. In eight (16%) patients of the Wallace group, 1–3 embryos were found in the fluid sucked from the external os, compared with three (6%) patients in the Erlangen group, but again this difference was not significant. In 92% of patients who became pregnant, the transfer procedure was smooth and easy. Successful embryo transfer was not influenced by the time of transfer post-insemination. The choice of catheter did not affect pregnancy rate. In cases in which transcervical transfer is very difficult or impossible, transvaginal–transmyometrial transfer is a viable option. The significance of early or late expulsion of transferred embryos into the vagina needs to be addressed in larger controlled studies.

Key words: embryo transfer/implantation/time of transfer/transfer catheter

Human Reproduction vol.14 no.3 pp.677–682, 1999

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Ovarian stimulation, oocyte retrieval and IVF

Ovarian stimulation was accomplished with follicle stimulating hormone (Metrodin; Serono, Rome, Italy) and human menopausal gonadotrophin (HMG) (Pergonal; Serono) after pituitary down-regulation with gonadotrophin-releasing hormone analogue (Decapeptyl; Beaufour Ipsen International, Paris, France). The dosage of HMG was adjusted individually according to the ovarian response. When at least three leading follicles had reached a mean diameter of ≥18 mm and serum oestradiol concentration was appropriate, human chorionic gonadotrophin (HCG) (10 000 IU; Pregnyl; Organon, Oss, The Netherlands) was administered intramuscularly (i.m.). Oocyte retrieval was scheduled to take place 34–36 h later by transvaginal ultrasound guidance. Six hours later the oocytes were inseminated and fertilization was confirmed by the presence of two pronuclei ~16 h after insemination. Preincubation, insemination and embryo culture were carried out in IVF media (Medi-cult a/s, Copenhagen, Denmark), details of which have been extensively described before (Ghazzawi et al., 1998).

Embryo development and morphology

On the morning of embryo transfer, embryos were examined and the number of cells determined. Each embryo was scored according to its symmetry and the extent of fragmentation of blastomeres (Plachot et al., 1986; Dawson et al., 1987; Scott et al., 1991). Briefly, grade I embryos contained symmetrical and unfragmented blastomeres, grade II embryos were even but with slight cellular debris and grade III embryos had at least one degenerated cell. Embryos were assigned to grade IV if three or more cells had completely fragmented. Embryos with the best morphology and the most advanced stage of development were selected for transfer. Normally up to three embryos were transferred in each group; however, in the event of slowly developing embryos in patients who were allocated for 48 h transfer, we had to delay the procedure a further 24 h.

Technique of embryo transfer

Patients were positioned supine (lithotomy position) on an electro-hydraulic gynaecological chair. The chair was tilted 20° to 30° from the horizontal so that the patient was in a head down position. The Erlangen catheter consisted of an introducing metal cannula (fitted with an obturator) and an insertion catheter. The cannula has an external diameter of 2 mm, and its tip is olive-shaped with a diameter of 3 mm. The silicon movable collar is usually placed 2 to 3 cm from the tip. The instrument has a length of 25 cm. To facilitate handling, the proximal end of the instrument is provided with a ring to accommodate the operator’s finger. The quality of the steel used for the instrument permits the cannula to be bent to match the individual ‘angle of kink’ between the uterine corpus and the cervix. We make use of an opened-tip vena cava catheter (17 guage) as the insertion catheter, the tip of which is slightly rounded. The outer diameter of the catheter is 1.2 mm and it bears two marks: one corresponding to the length of the introducing cannula, and the second located 4 cm proximal to the first. We first mark the catheter and then sterilize it using gas (ethylene oxide).

The Edwards–Wallace soft silicon catheter was preloaded with medium under a stereomicroscope to ensure that the embryos were actually released into the uterine cavity. The Edwards–Wallace soft silicon catheter was preloaded with embryos and then inserted through the Teflon sleeve, which was then passed through the external and internal cervical os following precise markings so that the embryos were placed into the uterine cavity and not into the fundus. The mark at the external os would correspond to the length of the cervical canal. Grasping the cervix was performed only when difficulty in introducing the catheter was encountered. From then onward, the same steps were followed as previously mentioned. In patients with difficult transfers, sounding the uterus (i.e. measurement of the uterine cavity length using a uterine sound instrument) and occasionally dilatation of the cervix was performed under light anaesthesia. Details of the transfer procedure including duration, bleeding, mucus and excessive manipulation were carefully recorded. Ease of use of catheters was measured by incidence of uterine sounding, cervical dilatation and bleeding. At the end of the procedure, the patient was carried to a bed where she remained for 1 h. Thereafter, she was discharged and allowed to take up her usual activities. Vaginal progesterone pessaries (Cyclopest; Hoechst, Frankfurt, Germany) supported the luteal phase.

Transmyometrial embryo transfer

In five patients (not included in the groups compared) with a history of repeated IVF failures, in whom embryo transfer was extremely difficult or even impossible, transvaginal–transmyometrial transfer was performed under ultrasound scan guidance. A 5 MHz probe (Hitachi 405) was inserted in the vagina and a ‘Towako’ embryo transfer catheter (Cook, Queensland, Australia) was used. The catheter 18-gauge needle with its stylet was passed under ultrasound guidance through the anterior fornix of the vagina and the myometrium of the anterior uterine wall. In one case, the uterus was retroverted, and the needle was passed through the posterior fornix and the posterior uterine wall. The needle was advanced through the myometrium to the junction with the endometrium without puncture of the latter. The stylet was then removed and the preloaded transfer catheter was passed through the needle. After release of the embryos, the catheter was checked in the laboratory to ensure that all embryos had been transferred.

Pregnancy was confirmed by an increased serum β-HCG concentration 14–16 days after embryo transfer. Clinical pregnancy was diagnosed by ultrasonography at 6–7 weeks of pregnancy.
Table I. Clinical characteristics of patients in both groups treated using different catheters

<table>
<thead>
<tr>
<th></th>
<th>Wallace</th>
<th>Erlangen</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cycles</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>144</td>
<td>142</td>
<td></td>
</tr>
<tr>
<td>Frozen</td>
<td>16</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td><strong>Age (years, mean ± SD)</strong></td>
<td>31.9 ± 4</td>
<td>31.2 ± 2.9</td>
<td></td>
</tr>
<tr>
<td><strong>Duration of infertility (years, mean ± SD)</strong></td>
<td>5 ± 1.5</td>
<td>4.7 ± 1.2</td>
<td></td>
</tr>
<tr>
<td><strong>No. of oocytes retrieved (mean ± SD)</strong></td>
<td>9.6 ± 3.3</td>
<td>9.3 ± 3.1</td>
<td></td>
</tr>
<tr>
<td><strong>No. embryos (mean ± SD)</strong></td>
<td>6.6 ± 2.5</td>
<td>6.2 ± 2.2</td>
<td></td>
</tr>
<tr>
<td><strong>Grade I + II embryos (n)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>432 (50)</td>
<td>426 (52)</td>
<td></td>
</tr>
<tr>
<td>Cryo-thawed</td>
<td>48 (41)</td>
<td>54 (43)</td>
<td></td>
</tr>
</tbody>
</table>

Values in parentheses are percentages.

Table II. Comparison of technical details of embryo transfer procedure and pregnancy rates

<table>
<thead>
<tr>
<th></th>
<th>Wallace</th>
<th>Erlangen</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Procedure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sounding</td>
<td>36 (23)</td>
<td>6 (4)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Dilatation</td>
<td>22 (14)</td>
<td>7 (4)</td>
<td>0.0064</td>
</tr>
<tr>
<td>Bleeding</td>
<td>39 (24)</td>
<td>6 (4)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mucus attached to tip of catheter</td>
<td>65 (41)</td>
<td>0</td>
<td>0.0001</td>
</tr>
<tr>
<td>Embryos left at tip of catheter</td>
<td>16 (10)</td>
<td>0</td>
<td>0.0071</td>
</tr>
<tr>
<td>No. of embryos transferred</td>
<td>2.9 ± 1.1</td>
<td>2.8 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Pregnancy rate per embryo transfer</td>
<td>31 (19)</td>
<td>48 (30)</td>
<td>0.3805</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages.

Data analysis
Statistical analysis of discrete variables was by χ² analysis with Fisher’s exact test where applicable. The differences were considered significant at a level of P < 0.05.

Results
A total of 160 patients was included in each group. The percentage of fresh to frozen cycles was almost similar in both groups. Also there was no difference in other parameters which may have influenced the outcome, such as age, duration of infertility, and number and grade of transferred embryos whether in fresh or frozen cycles (Table I). Table II compares some of the technical results of the two groups. The ease with which the Erlangen catheter was used compared with that of the Wallace catheter was reflected in a significantly (P < 0.0001) lower incidence of uterine sounding of cervical dilatation and occurrence of bleeding. Also there was a significant increase (P < 0.0001) in the presence of mucus attached to the tip of the Wallace catheter, and consequently the number of embryos found at its tip compared with that of the Erlangen catheter.

Table III compares the time of post-insemination transfer within the same group of patients. Some of the patients who were allocated to the 48 h transfer had to be delayed a further 24 h because of slowly dividing embryos. The pregnancy rate appeared to be slightly higher in patients who had their embryos transferred after 72 h than in those patients who had their embryos transferred after 48 h, but this difference was not significant in either group. Table IV shows the number of embryos that were found in the cervical fluid 15 min following embryo transfer. In eight (16%) patients, between one and three embryos were found in the Wallace group compared with three (6%) in the Erlangen group. This difference was not significant.

The clinical features of pregnancies are shown in Table V. In the majority of cycles (89%), fresh embryos were transferred. In most of the patients who became pregnant (92%), the embryo transfer procedure was smooth and easy.

Discussion
Embryo transfer is the last and least successful step in IVF. It is still an enigma why transfer is the most inefficient step. Implantation is a rather unknown and poorly understood

Table III. Pregnancy rate for patients having embryo transfer 48 h or 72 h post-insemination grouped by the type of catheter used for transfer

<table>
<thead>
<tr>
<th>Catheter</th>
<th>Hours post-insemination</th>
<th>Cell stage</th>
<th>Patients</th>
<th>Pregnancies (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wallace</td>
<td>48</td>
<td>3–4</td>
<td>74</td>
<td>13 (17.5)</td>
</tr>
<tr>
<td>Erlangen</td>
<td>48</td>
<td>3–4</td>
<td>71</td>
<td>18 (26)</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages. There are no significant differences.

Table IV. Embryos found in the fluid leaking from external os 15 min following embryo transfer

<table>
<thead>
<tr>
<th></th>
<th>No. of embryos</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wallace (n = 50)</td>
<td>1</td>
<td>3a</td>
</tr>
<tr>
<td>Erlangen (n = 50)</td>
<td>1</td>
<td>3b</td>
</tr>
</tbody>
</table>

aTotal no. of patients in this group = 8 (16%).
bTotal no. of patients in this group = 3 (6%).

Table V. Clinical features of pregnancies in the two catheter groups (n = 79)

<table>
<thead>
<tr>
<th></th>
<th>Wallace</th>
<th>Erlangen</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>27</td>
<td>43</td>
<td>70 (89)</td>
</tr>
<tr>
<td>Frozen</td>
<td>4</td>
<td>5</td>
<td>9 (11)</td>
</tr>
<tr>
<td>Embryo transfera</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Easy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difficult</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of gestational sacs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One gestational sac</td>
<td>63 (80)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two gestational sacs</td>
<td>15 (19)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Three gestational sacs</td>
<td>1 (1.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spontaneous abortions</td>
<td>12 (15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ongoing pregnanciesa</td>
<td>67 (85)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values in parentheses are percentages.

aData from the two catheter groups pooled.

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phenomenon in humans. There are no markers by which to evaluate implantation other than β-HCG concentration and the pregnancy-induced proteins. After embryo transfer, physicians and embryologists lose control of the cleaving embryos. One wonders whether all the embryos reach the endometrial cavity and whether all of them have the same chance of implantation. Implantation in humans is influenced by important variables such as the patient’s age, and the number and quality of the replaced embryos. In this study, we wanted to focus on other variables that might account for low implantation rates. The influence of the following embryo transfer conditions on implantation rate has been investigated: time of replaced embryos, the choice of catheter, the ease and difficulty of transfer procedure, and the significance of early or late expulsion of transferred embryos into the vagina.

**Time of embryo replacement**

It is generally agreed that the selection of embryos for transfer on the basis of morphology and development relates well to pregnancy outcome (Dawson et al., 1995). Embryos are usually transferred 2 days after insemination, after confirming fertilization and subsequent cleavage, at the 2- and 4-cell stages of development. Delaying embryo transfer until day 3 (when 6–8-cell stage of development is expected) provides an opportunity to observe the embryos for a further 24 h in culture. Any morphologically normal embryos on day 2 which subsequently arrest or degenerate can be identified and their transfer avoided. The embryo quality is unlikely to change between day 2 and day 3 (Dawson et al., 1995). In this study, the transfer of the more advanced growing embryos (day 3) achieved higher pregnancy rates than transfer on day 2 (Table III) but this difference was not significant. In a study by Gardner et al. (1998), a pregnancy rate of 70% was achieved by transferring the blastocysts on day 5 using sequential culture media in the absence of co-culture and serum. However, the true value of delaying transfer to beyond day 2 post-insemination can only be assessed using randomized prospective study with matched patient groups.

**The choice of catheter for embryo replacement**

Among the few essential steps of embryo transfer, catheter technology emerges as one crucial factor which has not gained enough attention and scrutiny. The aim must be to transfer the embryos with a high degree of reliabilityatraumatically, i.e. without any traces of blood on the introducing cannula and/or the insertion catheter. The operators’ experience plays an important role. In a study by Barber et al. (Barber et al., 1996), a comparable success rate was achieved when an infertility nurse performed the embryo transfer. Since 1994, we have preferred soft embryo transfer catheters to the more rigid ones, because the latter are more likely to induce cervical and endometrial lacerations. However, passing soft catheters through the cervical canal was often difficult and sometimes impossible. Soft catheters resulted in the highest rate (37.6%) of difficult embryo transfer with the consequences of lowering the pregnancy rate (Mansour et al., 1990). Failed embryo transfer and repeated attempts to replace the embryos using different catheters are very frustrating and might have adverse effects on the embryos. Therefore, the initial choice of transfer catheter is an important decision to make, and the physician must be comfortable and familiar with it.

The type of transfer catheter has been addressed in few studies. Wisanto et al. (Wisanto et al., 1989) recommended the use of the Frydman set over the Wallace catheter because of higher pregnancy rates. Gonen et al. (Gonen et al., 1991) has shown that the tef cat catheter yielded a higher pregnancy rate than the Frydman set. Al-Shawaf et al. (Al-Shawaf et al., 1993) showed that there was no difference in the performance of the Wallace and the Frydman catheters with regard to pregnancy rates (30.3% versus 30.7% respectively).

Taking into consideration that the above transfer catheters show few differences in concept and technology, these findings suggest that the issue of catheter type is still controversial and that there is no clear-cut advantage of one catheter over another. In the present study, we have investigated the use of a catheter which has major differences from the above traditional types of catheters. The Erlangen catheter was first introduced for clinical use in the early 1980s (Tronckow et al., 1983). Embryo transfer was carried out using the afterload method. Technically, various investigators handle embryo transfer in quite different ways (Lopata et al., 1980; Craft et al., 1981; Wood et al., 1981). There are various arguments in favour of the use of an insertion catheter as well as an introducing cannula. If only a single instrument is used, that is if the attempt is made to enter the uterine cavity only with the insertion catheter itself, it is not possible to exclude reliably an occasional haemorrhage or mucus while negotiating the cervical canal. In such a case, the possibility of the embryo coming into contact with blood, fibrin and endocervical microorganisms when passing the tip of the catheter after being expelled into the uterus is greater. In a study by Egbase et al. (Egbase et al., 1996), contamination of the embryo transfer catheter tip yielded lower pregnancy rates. If the two-instrument procedure is employed, i.e. the introducing cannula is fitted with an obturator during its introduction, these complications can largely be excluded. This has been reflected in our results.

In the group of patients who had embryo transfer by the Wallace catheter (Table II), bleeding occurred in 24% of them compared with 6% in the Erlangen catheter group. As we have mentioned above, a cannula with a fitted obturator probably clears the way for the insertion catheter. Therefore, there was hardly any mucus attached to the tip of the catheter upon withdrawal, while in 41% of the Wallace group, mucus was seen threading out from the tip of the catheter upon removal. Consequently, the number of embryos left at the tip of catheter that were trapped by blood or mucus was significantly higher (P < 0.007) in patients of the Wallace group than that of the Erlangen catheter group of patients. It is inevitable, therefore, that implantation is influenced by such mechanical factors that could hinder the proper placement of embryos into the uterine cavity.

**The ease and difficulty of transfer procedure**

Transcervical embryo transfer is the method used almost universally in human IVF programmes and has not been modified since the first report of successful attempts (Edwards,
Mechanical factors including the position of the patient during embryo transfer and bed rest following the procedure have been implicated as potential causes which could influence retention or expulsion of embryos following embryo transfer. Whereas our transfers were performed in the lithotomy position, other teams recommended that the patient’s position should depend on the position of the uterus. It has been suggested that women with a retroverted uterus should assume the knee-chest position, while a modified lithotomy position is performed for women with an anteverted uterus (Jones et al., 1983). Others suggested the modified lithotomy position for both these situations (Englert et al., 1986; Rienthaller et al., 1986). In a randomized study reported by Diedrich et al. (Diedrich et al., 1989), no difference in pregnancy rate was noted depending on the position during the transfer.

Historically, bed rest following embryo transfer has been generally advised, ranging in duration from 15 min to 24 h or more (Sharif et al., 1995). In a study by Botta and Grudzinkas (Botta and Grudzinkas, 1997), a 24 h period of bed rest following embryo transfer was not associated with a better outcome of the IVF when compared with that of a 20-min rest period.

The amount of back-tracking of embryos on withdrawal of the catheter has been a subject of debate. Woolcott and Stanger (Woolcott and Stanger, 1997) observed that embryos’ associated air were expelled upon withdrawal of the catheter, in 5% of all cases. However, when this did occur it was not insignificant, being <5 mm in all cases. This is contrary to the results published by Knutzen et al. (1992) who suggested that embryos might be expelled in 32–52% of embryo transfers depending on the patient’s position. Their study, however, investigated mock transfers with 40 ml of radio-opaque contrast medium in non-treatment cycles. The volume and nature of the contrast media differed considerably from those that are normally used during IVF treatment cycles. In another study by Schulman (Schulman, 1986), delayed expulsion was observed after seven transfers in 32 patients (22%); he concluded that his results were consistent with other evidence, not supported by direct observation, that expulsion of embryos may contribute to IVF failure. In the present study, 100 patients were subjected to another examination 15 min following embryo transfer. Delayed expulsion was noticed in 11 patients out of 100 transfers. However, there was no significant difference relating the type of catheter to expulsion rate. Whether we should routinely observe and examine any fluid leaking from the external os in order to establish early or late expulsion of transferred embryos needs to be addressed in larger controlled studies.

In conclusion, we have examined some of the factors related to embryo transfer conditions that may be of importance for achieving successful transfer and we have demonstrated that successful embryo transfer is not influenced by the time of transfer post-insemination. The role of the operator and his choice of embryo transfer catheter may influence catheter performance as reflected by pregnancy rate but no significant difference was observed in this study. In cases in which transcervical embryo transfer is very difficult or impossible, transvaginal–transcervical transfer is a viable option. The significance of early or late expulsion of transferred embryos into the vagina has yet to be established in large controlled studies.

References


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Received on April 16, 1998; accepted on November 18, 1998


Received on April 16, 1998; accepted on November 18, 1998

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