The technique for human embryo transfer

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In a program of human in vitro fertilization (IVF), the results of 204 attempted intracervical embryo transfers (ETs), using a variety of catheters in three trials over 18 months, have been analyzed for the ease of transfer and pregnancy rate. In nulliparous patients, transfers were more difficult than in multiparous patients; and a closed-end Teflon catheter was found to be more easily passed through the smaller cervical canal than an open-end catheter. The overall pregnancy rate was 17% (March 1980 to August 1981) and was not related to catheter type, although when chemical pregnancies were excluded, it was found that transfers using open-end catheters were more successful. The transfer procedure developed finally for routine use incorporates a consideration of these results. Fertil Steril 38:156, 1982

The success rate of in vitro fertilization (IVF) depends on maximizing the efficiency of each integral step of the procedure. In the past, effort was directed toward improving the methods for collecting oocytes in the natural and stimulated ovulatory cycles, the technique of oocyte recovery, and the rate of fertilization and embryo development. As a consequence, the rate of embryo development of oocytes obtained from patients at laparoscopy is very high and in the established clinics may approach 90%. However, the pregnancy rate of transferred embryos is relatively low, and even in the well-established clinics on the order of 15% to 20%.

Herein we report the results of all attempted embryo transfers (ETs) carried out over 18 months in which catheters of different sizes and construction were used in three trials to assess whether the type of catheter had any influence on the pregnancy rate following ET. The data were analyzed for the ease of transfer and proportion of pregnancies obtained with the use of each type of catheter.

MATERIALS AND METHODS

EXPERIMENTAL TRIALS

Nylon intravenous (IV) catheters (4 French gauge, external diameter 1.34 mm, Portex Ltd., Kent, England) were used for ET in an initial trial, but these were replaced by Teflon catheters in the second trial when experiments showed that mouse embryos were unable to survive incubation for 1 hour within the nylon catheters at 37°C. Mouse embryos were unaffected by similar incubation within the Teflon catheters.

In the second trial, a range of different gauge open-end catheters were used in a randomized sequence in an attempt to determine whether catheter size had an effect on the ease of transfer.
and success of establishing pregnancy. Teflon of 20 to 30 gauge (Table 1) was cut into 30-cm lengths and attached to blunted Luer needles. The catheters were washed for tissue culture and sterilized by dry heat. Embryos were drawn into the catheters in random lengths and attached to blunted Luer needles. The catheters were inserted into the cervical canal with the aid of sterile sponge forceps.

In the third trial, two different types of Teflon catheters (Fig. 1) were examined in random sequence. An open-end 22-gauge catheter with an internal diameter of 0.74 mm and an external diameter of 1.33 mm, with a polished end, was compared with a closed-end (bullet-shaped) catheter of similar dimensions with a side opening hole 0.634 mm in diameter (Fig. 1). Both catheters were 30 cm in length and were carried within an outer Teflon sleeve of 20 cm in length (Fig. 2). Both catheters were manufactured to specification by W. A. Cook Ltd., Surrey Hills, Victoria, Australia. The outer sleeve assists the delivery of the sterile inner catheter into the cervical canal and also assists any manipulation of the inner catheter within the cervical canal if some difficulty occurs in negotiating the internal os.

### PATIENTS

The study covers the period of March 1980 to September 1981, when 204 embryo transfers were carried out in patients whose preovulatory oocytes has been successfully fertilized in vitro. Three major clinical groups of patients were involved in the program. These were patients with blocked or damaged fallopian tubes, patients who failed to conceive after 12 months of artificial insemination with donor (AID) semen, and patients with infertility of unknown cause of at least 4 years’ duration. Of the 204 patients, 134 were nulliparae and 70 were multiparae. Patients were randomized for catheter type, although no difference in pregnancy rate between the groups has been found following transfer of embryos.8

### OPERATIVE PROCEDURE

With the exception of three patients who required epidural anesthesia to relax vaginal contraction, all ETs were carried out in the operating room without anesthesia. Oral Diazepam (10 mg, Roche Products Ltd., Sydney, Australia) was given 1 hour before transfer, and the patient’s husband and a nursing member of the team sat by the patient to help relieve anxiety. The patient was placed in slight Trendelenburg position. A gentle vaginal examination confirmed the position of the uterus, and an attempt was made to align the cervical canal by gentle manipulation of the uterus to allow the catheter to pass easily through the internal os, particularly in cases of marked anteversion or retroversion.

The cervix was exposed with a bivalve speculum, and the ectocervix was gently cleaned with two small swabs soaked in culture medium.5 The vulva and vagina were not prepared in any way, and the speculum was lubricated by sterile normal saline. Embryos may be transferred from the 2-cell through to 16-cell stages, although most embryos transferred in the present series were at either the 2- or 4-cell stage. Between one and three embryos were transferred, depending upon the number available. If more than three embryos developed, the excess number were deep frozen with a view to transfer at a later date.

### EMBRYO TRANSFER PROCEDURE

In trials 1 and 2, the embryos were loaded in the catheters by simply being drawn up in 20 to 35 μl of culture medium followed by 1 cm of air.
When the catheters were in position within the uterus, the embryos were expelled by injection of the volume taken to load the catheter and an additional 15 μl of air. The catheters were left undisturbed for 60 seconds to ensure the fluid and embryos were expelled from the catheter. Preliminary experiments showed that if mucus blocks the opening, particularly on catheters of a finer gauge, it may take 15 to 20 seconds for the fluid to escape from the catheter.

The transfer procedure used in the third trial was as follows:

1. A 1-ml syringe and the inner catheter were attached (Fig. 3) and completely filled with culture medium.
2. The syringe was set at 0.2 ml, and 1 cm of air was drawn into the tip of the catheter.
3. The embryos were drawn up in 20 to 35 μl of culture medium.
4. A further 1-cm air space was drawn into the tip of the catheter (Fig. 1).
5. The loaded catheter was brought quickly from the laboratory to the operating room, where it was inserted into the outer sleeve, which was then placed gently into the external cervical os.
6. The internal catheter was passed slowly and gently along the sleeve through the cervical canal into the uterine cavity to within 5 mm of the fundus. This distance was accurately measured on the distal end of the catheter prior to transfer, with the use of the information of uterine cavity length previously measured by ultrasound (Fig. 3).
7. When the transfer catheter was in the correct position, the embryos were expelled by depressing the 1-ml syringe to 0.18 ml. The catheter was left undisturbed for 60 seconds, after which time a further 20 μl of culture medium was injected to ensure that the embryos had not returned to the catheter.

The catheter was slowly withdrawn from the uterus and promptly checked under the microscope to ensure that the embryo had not been inadvertently retained. The external surface of the catheter and external os were also checked for any sign of blood, mucus, or culture medium.

If there was difficulty in passing the catheter through the internal os, in some cases the more rigid outer sleeve was advanced through the os.

RESULTS

EASY TRANSFERS

No difficulty was encountered in this group. Embryos were delivered into the uterine cavity at the first attempt, and little or no mucus was found on the catheter.

SUSPECT TRANSFERS

There was either some difficulty passing the internal os or uncertainty of the position of the tip of the catheter. This group also included the appearance of blood or fluid on the catheter or internal os.

DIFFICULT TRANSFERS

These involved long periods of manipulations to pass the catheter through the cervix. Some force was often needed, and some bleeding was usually seen.

There were 204 ETs carried out during a period of 18 months, resulting in 34 pregnancies, 12 of which were diagnosed by a significant period of amenorrhea with rising levels of beta human chorionic gonadotropin (β-hCG). All 34 pregnancies occurred in the easy transfer group.

The ease of transfer was not affected by the design or material of the catheter; the diameter was unimportant unless it was very fine (30-gauge), in which case transfer was difficult (Table 2).

The overall pregnancy rate was not affected by the design of catheter. If hCG rises were excluded, the pregnancy rate was higher with the open-end catheter.

The correct placement of the catheter in the uterine lumen depends on previous assessment or ultrasound measurement of the uterine cavity and cervical canal length (y), which may be used to determine how far the internal catheter should protrude (x) from the end of the external catheter located at the external os. Embryos are expelled 5 mm from the fundus, and the distance x may be measured at the end of the internal catheter with the syringe attachment.
The effects of parity on the transfer results are shown in Table 3. The closed-end catheter had significantly fewer transfer failures than the open-end catheter in nulliparous patients (binomial test, $P = 0.01$), but the open-end catheter was connected with a higher pregnancy rate than the closed-end catheter in multiparous patients (binomial test, $P = 0.03$).

DISCUSSION

The essential requirements for ET are simplicity and effectiveness in the establishment of pregnancy. The nonsurgical cervical approach for ET described in this paper is rapid and simple and requires no anesthesia. The surgical transfer procedure that entails the catheterization of the uterine lumen through the uterine wall is the normal practice for transfer of animal embryos, including primates. In women the uterine wall is thick and generally more difficult to penetrate than in most animal species, and surgical transfers are complicated by some serosal bleeding.

It is possible that factors other than the transfer technique may govern the success rate of ET, in particular the viability or potential for development of the embryos produced by in vitro fertilization. If this is the case, transfer of embryos by surgical methods may not greatly increase the pregnancy rate. The transcervical approach has the potential problem of introducing infection into the uterus from the cervical canal and may also carry mucus or blood into the uterine lumen, which may interfere with embryo development and implantation.

In cattle the transcervical route for ET is not as efficient as the surgical implantation of embryos, although reasonable pregnancy rates may be achieved by careful procedures and transfer of more advanced embryo stages. In cattle it is thought that increased uterine activity and sensitivity to manipulation around the time of ovulation and the 3 to 4 days immediately after results in the expulsion of embryos from the uterus. In our own studies in women the administration of antiprostaglandins to reduce uterine contractility had no effect on improving pregnancy rates. In addition, treatment of patients with progesterone (P) before or after ET had no beneficial effect on the pregnancy rate. The outer sheath may protect the inner catheter from contamination by microorganisms in the vagina and

Table 2: Trial Investigating Catheter Type for Embryo Transfer

<table>
<thead>
<tr>
<th>Catheter type</th>
<th>Easy (%)</th>
<th>Subject</th>
<th>Difficult</th>
<th>Failed</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open-end nylon</td>
<td>7 (70)</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>20-gauge Teflon</td>
<td>11 (79)</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>22-gauge Teflon</td>
<td>16 (78)</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>26-gauge Teflon</td>
<td>26 (69)</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>30</td>
</tr>
<tr>
<td>Open-end Teflon</td>
<td>22 (84)</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td>Closed-end Teflon</td>
<td>40 (91)</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td>Total</td>
<td>159 (78)</td>
<td>16</td>
<td>12</td>
<td>17</td>
<td>204</td>
</tr>
</tbody>
</table>

*Transfer succeeded when a closed-end catheter was used in one case, and transfundal transfer under anesthesia was required in the other case.

$\chi^2$ analysis. NS, not significant; S, significant.
Table 3. Effect of Parity on the Ease and Success of Embryo Transfer

<table>
<thead>
<tr>
<th>Catheter</th>
<th>Parity</th>
<th>Difficulty of transfer</th>
<th>Result of transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Easy</td>
<td>Suspect</td>
</tr>
<tr>
<td>Open</td>
<td>Nulliparous</td>
<td>70</td>
<td>10</td>
</tr>
<tr>
<td>Open</td>
<td>Multiparous</td>
<td>49</td>
<td>4</td>
</tr>
<tr>
<td>Closed</td>
<td>Nulliparous</td>
<td>27</td>
<td>1</td>
</tr>
<tr>
<td>Closed</td>
<td>Multiparous</td>
<td>13</td>
<td>1</td>
</tr>
</tbody>
</table>

reduce the chance of intrauterine infection. This principle has been advocated in transcervical ET in cattle.\(^\text{11}\)

Few descriptions of human ETs have been published. In 1973 our group described the transfer of an 8-cell embryo using a plastic cannula inserted into the uterus through which was inserted a fine polyethylene catheter containing the embryo in 0.2 ml culture medium.\(^\text{14}\) A similar technique was described by these authors in 1978 when three embryos were transferred to the uterus by a transparent polyethylene catheter via the cervix without an accompanying sleeve, although no pregnancies were obtained.\(^\text{15}\)

Steptoe et al.\(^\text{16}\) describe the ET technique culminating in their first successful live birth. A polyethylene cannula 2.2 mm in external diameter was placed through the cervical canal, and a narrower catheter, 1.3 mm in diameter, containing the embryo and culture medium was threaded within the larger one. After transfer, both cannulas were withdrawn simultaneously. These authors also stress the need for checking the catheter following transfer for a retained embryo. This problem has not occurred in the present series of experiments, with the exception of damage to one embryo when Rampley's sponge-holding forceps crushed the catheter during transfer.

Lopata et al.\(^\text{17}\) described the ET technique resulting in a single live birth. A curved metal cannula with an inner diameter of 1.7 mm and an olive tip 3 mm in diameter was passed through the internal os. A fine nylon catheter (Portex Ltd., Kent, England) was then threaded inside the metal catheter to the uterine fundus, after which the metal cannula was completely withdrawn. We have not used this instrument, because its size and rigidity usually causes bleeding, and we consider the nylon catheters potentially toxic to human embryos.

Steptoe and Edwards recommend complete tubal occlusion\(^\text{18}\) before ET is attempted in cases of tubal infertility, because of the potential risk of tubal pregnancy, a case of which they described in 1976.\(^\text{19}\) We do not believe this prerequisite is needed. The potential risk of tubal pregnancy is negligible if a small volume of culture fluid is used and the catheter tip is accurately positioned in the uterus. No ectopic pregnancies have occurred in this series.

The present results demonstrate that pregnancy occurs only when a morphologically normal embryo is placed in the uterus following an easy transfer. The problems with the present technique are that the catheter picks up mucus during its passage through the cervix and sometimes catches in folds of cervical endothelium, especially around the internal os, causing delay and occasional bleeding. Analysis of the results of the two types of catheter show no significant differences in overall pregnancy rates and ease of transfer. However, in nulliparous patients, who have narrower and more tortuous cervical canals than multiparous patients, we found it easier to pass the closed-end catheter through the canal than the open-end catheter. This may be related to the capacity of the bullet-shaped end to pass more readily through a narrow opening at the internal os. When chemical or β-hCG rises were excluded from pregnancy rate analysis, it appeared that the open-end catheter might be more effective for establishing pregnancy, but this needs to be confirmed. It may be that the small exit hole in the closed-end catheter may be a disadvantage in the proper expulsion of the embryos, particularly if mucus is attached to the end of the catheter.

As a result of these findings, transfer is now attempted initially with the open-end catheter, and the closed-end catheter is used when difficulty is encountered in entering the uterine cavity or where previous transfer attempts have been consistently difficult.

The transcervical catheter route invariably disturbs the cervical mucus plug, creating an exit tract, possibly causing bleeding, and increasing uterine activity. We have, therefore, attempted the transfundal approach on three occasions, passing the catheter through a needle inserted into the uterine cavity at the time of laparoscopy. This method has the disadvantages of requiring...
general anesthesia and introducing some blood into the uterine cavity. No pregnancy resulted from these three attempts.

REFERENCES