Impact of Technical Difficulties, Choice of Catheter, and the Presence of Blood on the Success of Embryo Transfer—Experience from a Single Provider

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Purpose: To investigate the impact of technical difficulties, choice of catheter, and the presence of blood during embryo transfer on the results of in vitro fertilization and ICSI.

Methods: A cohort of 784 consecutive cycles in 655 in vitro fertilization and ICSI patients were studied.

Results: Negotiating the cervix, using the volsellum, presence of blood on the catheter wall or on the cervix did not affect the results. Changing the catheter and blood on the catheter tip reduced the pregnancy ($P < 0.05$ and $P < 0.05$) and implantation rates ($P < 0.001$ and $P < 0.01$). The Ultrasoft catheter produced higher pregnancy ($P < 0.0005$) and implantation rates ($P < 0.01$) compared to the more rigid Frydman catheter.

Conclusions: Negotiation of the cervix, the use of a volsellum, and the presence of blood on the catheter wall or on the cervix do not affect the results. Changing the catheter and blood on the catheter tip significantly diminish the pregnancy and implantation rates. Soft catheters perform better.

KEY WORDS: Clinical pregnancy rate; embryo transfer; implantation; implantation rate; in vitro fertilization.

INTRODUCTION

Embryo transfer (ET) is an integral part of in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). However, it is also the least successful and it has been estimated that 85% of the replaced embryos do not implant (1). This may be due to defects in the ET technique, inadequate endometrial receptivity, or the inability of the embryo to implant properly.

The relationship between difficult ETs and the clinical pregnancy (CPR) and implantation rates (IR) is still controversial. For example, Nabi *et al.* found

that the embryos were significantly more likely to be retained inside the ET catheter when the transfer procedure was difficult compared with when the transfer was easy (2). Similarly, Abusheikha *et al.* found that the CPR was significantly lower in difficult ETs compared to easy transfers (3). On the contrary, some studies found no relationship between the difficulty of ET and the CPR. For example, Burke *et al.* analyzed different variables present at the time of ET and found that the difficulty of the ET technique did not affect the CPR (4). Similarly, Tur-Kaspa *et al.* found no statistically significant differences in CPR between their easy and difficult transfers (5).

Various techniques performed during ET have been suspected of affecting the CPR and IR. For example, the use of a volsellum (tenaculum) to hold the cervix during ET was found to release oxytocin (6) and to stimulate uterine junctional zone contractions

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(7). However, the exact effect of this practice on the CPR and IR is not known. Performing the ET in two consecutive steps, if some or all of the embryos are retained in the transfer catheter (repeat transfers), has also been blamed for diminished CPRs by some authors (8). However, other studies found no significant difference in the CPR between the patients who had all their embryos transferred at the first attempt and those who required more than one attempt (2,5).

The presence of blood outside the catheter (but not inside) during ET has also been found to reduce the CPR and IR (9). Other authors reported that the embryos were significantly more likely to be retained when the ET catheter was contaminated with blood (2).

The effect of the type of catheter used in ET on the CPR and IR is similarly controversial. For example, Gonen *et al.* found that the Tomcat catheter yielded a significantly higher CPR than the Frydman catheter (10). Similarly, Meriano *et al.* found that the use of the Tomcat catheter resulted in significantly higher IR and CPR compared with that of TDT catheter (11). On the contrary, Burke *et al.* analyzed different variables present at the time of ET and found that the type of ET catheter did not affect the CPR. The rigidity of the catheter has also been found to affect the CPR and IR, but the results of different studies are also controversial. Some studies have reported that soft ET catheters were associated with higher CPR and IR compared to rigid catheters (12). Other studies found that the CPR was higher with rigid compared to soft catheters (13), while a third group of studies found no significant difference in the CPR between the soft and the rigid catheters (14–16).

Moreover, in most of these studies, more than one clinician was involved in the ET procedure. A recent study has reported a wide variation in CPR (17.0% vs. 54.3%, $P < 0.05$) between various providers performing ET in the same IVF programme (17).

The aim of this study was to analyze the following factors associated with ET, when ET is performed by the same provider, to further explore their impact on the CPR and IR: the difficulties encountered during ET, the presence of blood during ET, and the choice of the ET catheter.

MATERIALS AND METHODS

Patients

A total of 784 consecutive treatment cycles in 655 patients, attending our unit for IVF and ICSI

Table I. Criteria of the Studied Patients

Criterion	Value
Number of patients	655
Number of cycles	784
IVF cycles	195
ICSI cycles	589
Mean (SD) age of patients (years)	32.28 (5.72)
Mean (SD) age of husbands (years)	39.51 (17.63)
Mean (SD) duration of infertility (years)	7.77 (4.97)
Mean (SD) number of HMG ampoules	30.35 (7.94)
Mean number (SD) of oocytes retrieved	9.39 (6.93)
Mean number (SD) of metaphase II oocytes	6.85 (5.3)
Mean number (SD) of embryos obtained	5.57 (5.25)
Mean number (SD) of embryos replaced	2.71 (1.14)
Mean (SD) midluteal plasma progesterone (ng/mL)	46.21 (32.07)
Number of pregnancies (clinical pregnancy rate)	165 (21.05%)
Number of implanted embryos (implantation rate)	259 (12.19%)
Number of ectopic pregnancies (%)	4 (0.51%)

treatment from 1 January 1999 until 15 September 2001, were studied by retrospective analysis (Table I). Of these, 195 were IVF treatment cycles and 589 were ICSI treatment cycles. Cycles from patients who received cryopreserved embryos or who requested general anaesthesia were excluded from the study.

Ovarian Stimulation Protocol

Controlled ovarian stimulation was achieved by a triptorelin (Ferring, Kiel, Germany) short down-regulation protocol followed by human menopausal gonadotropin stimulation (Pergonal, Serono, Switzerland or Humegon, Organon, The Netherlands). Monitoring was effected by serial ultrasound scanning and hCG (10,000 IU) was administered by intramuscular injection (Profasi, Serono, Switzerland) when the leading follicle reached 18 mm in diameter, as described elsewhere (18). Oocyte retrieval was performed 36 h afterwards through the transvaginal ultrasound-guided route (Combison 310; Kretztechnik AG, Zipf, Austria). The IVF and ICSI procedures were carried out as described by Edwards *et al.* and Palermo *et al.*, respectively (19,20).

Embryo Transfer Procedure

Embryos were transferred 48 h after retrieval. Only class I and II embryos were replaced. All transfers were performed by the same clinician (HNS) with the patients in the lithotomy position. The procedure

was performed under sterile conditions; the patient was draped, a sterile speculum was inserted in the vagina, and the cervix exposed. The cervical mucus was aspirated using a mucus aspirator and the cervix was then cleansed with a swab soaked with culture medium (BM9 medium; CCD, Paris).

Embryo transfer was carried out using preloading- or after-loading-type catheters depending on the availability of the catheters and the results of a trial ET test performed on the initial visit of the patient. Four types of catheters were used during the study: (a) a rigid preloading-type catheter (Frydman; CCD, Paris), (b) a rigid after-loading-type catheter with an obturator and a soft inner catheter (TDT; CCD, Paris), (c) a ball-pointed rigid after-loading catheter with a soft inner catheter (Labotect; Labor-Technik, Göttingen, Germany, and (d) an after-loading-type catheter with an obturator and an inner ultrasoft catheter (Ultrasoft; CCD, Paris, France).

With the after-loading type catheters (TDT, Labotect, and Ultrasoft), the outer sheath with its obturator was passed first through the cervix until 1 cm above the internal cervical os. Once the outer sheath was in place, the embryologist (AFR or AFA) was informed. The inner soft catheter was then fitted with a 1 mL tuberculin-type syringe and flushed with BM9 medium. The embryos were then loaded at the distal end of the inner catheter in a volume of 15–25 μ L of BM9 medium, preceded and followed by 1 cm of air. The inner catheter containing the embryos was then advanced inside the outer catheter to 1 cm below the uterine fundus, according to the uterine length obtained by sounding at the initial visit of the patient. The embryos were then gently deposited into the uterine cavity.

The preloading-type catheter (Frydman) was fitted with a 1 mL tuberculin-type syringe and flushed with BM9 medium, just as done in the case of the after-loading type catheter. The embryos were then loaded by the embryologist at the distal end of the catheter again as described earlier, and brought to the clinician. The catheter was then advanced inside the uterine cavity by the clinician and the embryos were deposited gently as described earlier.

In all cases, the catheter was left in situ for 30–60 s and then withdrawn gently. It was then checked under a dissecting microscope for retained embryos. If these were found, they were reloaded and transferred again (repeat transfer). The patients were asked to remain in bed for 15–30 min.

If the ET could not be performed by the originally intended catheter, the embryos were reloaded

into the ball-pointed Labotect catheter. In one patient, transcervical ET was still impossible to perform and transvaginal transmyometrial ultrasound-directed ET was performed.

The details of the ET technique were registered immediately after the procedure, paying particular attention to the following:

1. Difficulties encountered during ET. These were classified as follows: (a) easy transfers in which the catheter entered the uterine cavity smoothly on the first attempt, (b) the need to negotiate the cervical canal during transfer, (c) the need to hold the cervix with a volsellum (tenaculum) during transfer, (d) the need to change the catheter to the ball-pointed Labotect catheter because of failure of introducing the original catheter, or (e) the necessity to carry on the transfer in two consecutive attempts (repeat transfers) because of the retention of some or all of the embryos in the catheter in the first attempt.
2. The presence of blood during ET. This was classified as follows: (a) no blood observed during ET, (b) presence of blood on the outer wall of the catheter, (c) presence of blood on the tip of the catheter, and (d) blood observed at the uterine cervix during ET or after withdrawal of the cannula.
3. The type of catheter used (Frydman, TDT, Labotect, or Ultrasoft).

In all cases, luteal-phase support was affected by oral administration of micronized progesterone 600 mg/day (Utrogestan; Laboratoires Bessins-Iscovesco, Paris, France) in three divided doses. The serum β -hCG concentration was measured 2 weeks after ET to determine the occurrence of pregnancy. Clinical pregnancy was defined as the observation of a fetus with a pulsating heart at 6–8 weeks after ET. The implantation rate was defined as the number of sacs seen on ultrasound in relation to the number of embryos replaced.

Data Analysis

Statistical analysis was performed using the Microstat software package. The chi-square test, the Fisher's exact test, and the chi-square test for independence for more than two groups were used to compare qualitative variables, and the Student's *t*-test to compare quantitative variables, as appropriate. The significance level was set at $P = 0.05$.

RESULTS

Overall Results

Transcervical ET was successful in 783 out of the 784 cycles studied. Difficulties were encountered in 149 instances (19.01%), while 635 transfers were classified as easy (80.99%). In one patient, transcervical ET could not be performed despite using a Labotect ball-point catheter and general anesthesia. Transvaginal transmyometrial ET was then performed, but the patient did not become pregnant. Out of the 783 transcervical ETs, blood was observed in 236 instances (30.14%) while 547 ETs were bloodless (69.86%). Out of the 784 cycles studied, 165 clinical pregnancies resulted, a CPR of 21.05%. A total of 2124 embryos were replaced (a mean of 2.71 embryos per cycle) and 259 implanted giving an IR of 12.19%. There were four ectopic pregnancies (0.51%).

Difficulties Encountered During ET

In the group of 149 difficult transfers, 23 clinical pregnancies resulted, a CPR of 15.44%. In comparison there were 142 pregnancies in the group of 635 easy transfers, a CPR of 22.36%. This difference did not reach statistical significance ($\chi^2 = 3.484$, $P = 0.0620$). However, in order to reach statistical significance, the least number needed to treat (NNT) was calculated. This was found to be 1235 cycles in each arm of the study, in order to increase the CPR from 20 to 25%, accepting a 90% probability of detecting a true difference and taking 5% as the level of significance. On the contrary, 222 embryos implanted out of

the 1703 replaced in the easy transfer group, an IR of 13.04% compared to 37 embryos out of 421 embryos replaced in the difficult transfer group, an IR of 9.27%. This difference is statistically significant ($\chi^2 = 5.687$, $P < 0.05$). The odds ratio (OR) for diminishing the IR were 0.64 (95% CI 0.56 – 0.73).

The CPR and IR associated with various difficulties encountered during ET are shown in Table II. Compared to easy transfers there were no statistically significant differences in CPR or IR, when the cervical canal was negotiated ($\chi^2 = 0.259$, $P = 0.6106$; $\chi^2 = 0.352$, $P = 0.5528$, respectively) or when the volsellum was used ($\chi^2 = 0.627$, $P = 0.4285$; $\chi^2 = 0.007$, $P = 0.9325$, respectively). Also there were no statistically significant differences in the CPR or IR between easy transfers and repeated transfers ($\chi^2 = 2.010$, $P = 0.1563$; $\chi^2 = 3.291$, $P = 0.096$, respectively). On the contrary, compared to easy transfers, the CPR and IR were significantly lower when the catheter was changed ($\chi^2 = 3.906$, $P < 0.05$; $\chi^2 = 10.859$, $P < 0.001$, respectively). The OR for reducing the CPR and IR when the catheter was changed compared to easy transfers were 0.26 (95% CI 0.08 – 0.83) and 0.13 (95% CI 0.03–0.53), respectively (Table III). Blood was present during ET (69.0%) in 20 cycles out of the 29 where the catheter was changed.

Presence of Blood During ET

In the group of 547 bloodless transfers, 126 pregnancies occurred, a CPR of 23.03%. In comparison there were 39 pregnancies in the 236 ETs in which blood was found, a CPR of 16.53%. This difference

Table II. Clinical Pregnancy (CPR) and Implantation (IR) Rates with Various Factors Affecting Embryo Transfer

	Pregnancies/transfers	CPR (%)	Implanted/transferred embryos	IR (%)
Easy transfers	142/635	22.36	222/1703	13.04
Negotiation of cervix	14/71	19.72	22/191	11.52
Use of volsellum	7/41	17.07	13/102	12.75
Change of catheter	2/29	6.89	2/102	1.96
Repeat transfers	0/7	0	0/22	0
Transmyometrial transfer	0/1	0	0/4	0
All difficult transfers	23/149	15.44	37/421	9.27
Bloodless transfers	126/547	23.03	195/1478	13.19
Blood on catheter wall	30/153	19.61	51/428	11.91
Blood on tip of catheter	7/66	10.61	10/164	6.1
Blood from cervix	2/17	11.76	3/50	6
All bloody transfers	39/236	16.53	64/642	9.97
Frydman catheter	12/98	12.24	18/195	9.23
TDT catheter	107/530	20.19	168/1476	11.38
Labotect catheter	24/97	24.74	40/275	14.55
Ultrasoft catheter	22/59	37.29	33/178	18.54
Total	165/784	21.05	259/2124	12.19

Table III. Odds Ratio (OR) and 95% Confidence Intervals (CI) for the Clinical Pregnancy (CPR) and Implantation Rates (IR) with Various Factors Affecting Embryo Transfer

	OR (CI) for CPR	OR (CI) for IR
Cervix negotiation vs. easy transfer	0.85 (0.46–1.56)	0.87 (0.54–1.39)
Volsellum vs. easy transfer	0.71 (0.31–1.63)	0.97 (0.53–1.77)
Catheter change vs. easy transfer	0.26 (0.08–0.83) ^a	0.13 (0.03–0.53) ^b
Difficult vs. easy transfers	0.63 (0.39–1.02)	0.64 (0.56–0.73) ^a
Blood on catheter wall vs. no blood	0.81 (0.52–1.27)	0.89 (0.64–1.24)
Blood on catheter tip vs. no blood	0.40 (0.18–0.90) ^a	0.43 (0.22–0.83) ^c
Blood from cervix vs. no blood	0.45 (0.10–2.00)	0.42 (0.13–1.37)
Bloody transfers vs. no blood	0.66 (0.44–0.98) ^a	0.73 (0.55–0.98) ^a
TDT vs. Frydman catheter	1.81 (0.95–3.43)	1.26 (0.76–2.10)
Labotect vs. Frydman catheter	2.36 (1.23–4.52) ^a	1.67 (0.93–3.01)
Labotect vs. TDT catheter	1.30 (0.78–2.16)	1.33 (0.92–1.93)
Ultrasoft vs. Frydman catheter	4.26 (1.91–9.48) ^d	2.24 (1.22–4.12) ^c
Ultrasoft vs. TDT catheter	2.35 (1.33–4.16) ^e	1.77 (1.17–2.67) ^c
Ultrasoft vs. Labotect catheter	1.81 (0.90–3.64)	1.34 (0.80–2.23)

^a Statistically significant ($p < 0.05$).^b Statistically significant ($p < 0.001$).^c Statistically significant ($p < 0.01$).^d Statistically significant ($p < 0.0005$).^e Statistically significant ($p < 0.005$).

is statistically significant ($\chi^2 = 4.200$, $P < 0.05$). The OR for diminishing the CPR were 0.66 (95% CI 0.44–0.98). A total of 195 embryos implanted out of 1478 replaced in the bloodless ET cycles, an IR of 13.19%. In comparison, 64 embryos out of 642 implanted in the cycles with bloody ETs, an IR of 9.97%. This difference is also statistically significant ($\chi^2 = 4.340$, $P < 0.05$). The odds ratio (OR) for diminishing the IR were 0.73 (95% CI 0.55–0.98).

The CPR and IR associated with different instances of blood during transfer are shown in Table II. Compared to bloodless transfers there were no statistically significant differences in CPR or IR, when blood was present on the outer wall of the catheter ($\chi^2 = 0.792$, $P = 0.3735$; $\chi^2 = 0.457$, $P = 0.4992$, respectively). Similarly, compared to bloodless transfers, there were no statistically significant differences in CPR or IR, when blood was seen at the cervix ($\chi^2 = 1.186$, $P = 0.2761$; $\chi^2 = 2.202$, $P = 0.1378$, respectively). On the contrary, compared to bloodless transfers, the CPR and IR were significantly lower when blood was present on the tip of the catheter ($\chi^2 = 5.355$, $P < 0.05$; $\chi^2 = 6.803$, $P < 0.01$, respectively). The OR for reducing the CPR and IR were 0.40 (95% CI 0.18–0.90) and 0.43 (95% CI 0.22–0.83), respectively (Table III).

Type of Catheter Used in ET

The Frydman catheter was used in 98 instances (12.5%), the TDT catheter in 530 instances (67.6%),

the Labotect catheter in 97 instances (12.5%), and the Ultrasoft catheter in 59 instances (7.5%).

The CPR and IR associated with each catheter are shown in Table II. The chi-square test for independence with more than two groups revealed that there was a statistically significant difference in CPR between the four catheters used ($\chi^2 = 14.967$, $P < 0.005$). Similarly, there was a statistically significant difference in IR between the four catheters used ($\chi^2 = 11.881$, $P < 0.01$). However, there were no statistically significant differences in CPR or IR, between using the TDT or the Frydman catheter ($\chi^2 = 3.398$, $P = 0.0653$; $\chi^2 = 0.806$, $P = 0.3693$, respectively). On the contrary, the CPR was significantly higher when the Labotect catheter was used compared to the Frydman catheter ($\chi^2 = 5.058$, $P < 0.05$). The OR for the increase in CPR was 2.36 (95% CI 1.23–4.52). The IR was also higher, but the difference was not statistically significant ($\chi^2 = 2.279$, $P = 0.0843$). Both the CPR and IR were significantly higher when the Ultrasoft catheter was used compared to the Frydman catheter ($\chi^2 = 13.614$, $P < 0.0005$; $\chi^2 = 6.831$, $P < 0.01$, respectively). The ORs for the increase in CPR and IR were 4.26 (95% CI 1.91–9.48) and 2.24 (95% CI 1.22–4.12), respectively. The CPR and IR were also significantly higher with the Ultrasoft catheter group compared to the TDT catheter ($\chi^2 = 9.075$, $P < 0.005$; $\chi^2 = 7.622$, $P < 0.01$, respectively). The OR for the increase in CPR and IR were 2.35 (95% CI 1.33–4.16) and 1.77 (95% CI 1.17–2.67), respectively (Table III). There were no statistically significant

differences in CPR or IR between the use of the Ultrasoft and Labotect catheters ($\chi^2 = 2.777$, $P = 0.0956$ and $\chi^2 = 1.275$, $P = 0.2588$, respectively).

Ectopic Pregnancies

There were four ectopic pregnancies (0.51%). The TDT catheter was used in all of them. In three cases, blood was present on the outer wall of the ET catheter. In three cases, the ET was easy and in one case, negotiation of the cervix was performed during ET.

DISCUSSION

Difficult ET has been suspected of affecting the results of IVF and ICSI since the introduction of these techniques (21,22). However, recent studies have reported that although the CPR and/or the IR were diminished in difficult transfers compared to easy transfer, the differences were not statistically significant (5,23). It has been suggested that more powerful studies may yield different results and the fact that ET is performed by various clinicians may also confound the findings. In a recent study, Hearn-Stokes *et al.* (15) evaluated the effect of individual providers on pregnancy outcome after ET and found that the CPR varied significantly between clinicians performing the ET step (17.0% vs. 54.3%, $P < 0.05$). In the present study, all the ETs were performed by the same clinician.

The results of the present study show that, analyzed collectively, difficult transfers were associated with a lower CPR compared to easy transfers, but the difference did not reach statistical significance ($P = 0.0620$). On the contrary, we have found that the IR was significantly reduced by about one third in difficult transfers compared to easy transfers. Unfortunately, many studies did not report the effect of difficult transfers on the IR (4,5,23). Our findings suggest that it may be advantageous to increase the number of embryos replaced if a difficult ET is anticipated.

Detailed analysis of our data showed that the necessity of negotiating the cervical canal with the ET catheter did not affect the CPR or IR compared to easy transfers. Similarly, holding the cervix with the volsellum did not affect the CPR or IR. This practice has been previously shown to be associated with release of oxytocin (6) and to initiate junctional zone uterine contractions (7), but these two studies did not report on its effects on the CPR or IR. Our findings

suggest that these contractions may not necessarily be detrimental to ET.

We had no pregnancies in the seven cycles where the embryos were replaced in two consecutive attempts (repeat transfers) compared to easy transfers. These findings agree with those of Burke *et al.*, who found that the two most important variables for predicting a clinical pregnancy were a first-time transfer and the number of high-grade embryos replaced (4); but disagree with those of Nabi *et al.* and Tur-Kaspa *et al.* (2,5), who found no statistically significant differences between first-attempt transfers and repeat transfers. This discrepancy in the findings may reflect the necessity of studying larger number of cycles. Similarly, we have found that changing the catheter, after failure of the original catheter to enter the uterine cavity resulted in a significant reduction in both the CPR and IR. This is probably due to injury to the endometrium as 69% of these transfers were associated with blood. Changing the catheter was necessary in these cases despite our practice of performing a trial ET on the initial visit of the patient. It may therefore be necessary to carry out the trial ET on more than one occasion in the same patient.

We have found that the presence of blood during ET significantly diminishes the CPR and IR by about one third. This confirms the findings of Goudas *et al.* (9) and stresses the necessity of a gentle and atraumatic technique. We have found that the presence of blood on the outer wall of the catheter did not affect the CPR or IR. However, the presence of blood on the tip of the catheter significantly reduced the CPR and IR probably denoting that the tip of the catheter accidentally indented the endometrium.

We have also found that the soft after-loading catheter (Ultrasoft) was associated with a fourfold increase in CPR and a twofold increase in IR compared to the more rigid preloading Frydman catheter. It was also associated with a twofold increase in CPR and IR compared to the more rigid after-loading TDT catheter. This is probably due to less trauma being inflicted by the soft catheter on the endometrium. Our findings confirm those reported by Wood *et al.* (12), although their work was confounded by the use of ultrasound-guidance besides using a soft catheter. They disagree with the smaller studies that found no difference between the use of the soft (Wallace) catheter and the more rigid (Frydman) catheter (13–16), again reflecting the necessity of more powerful studies. In our study, the Labotect catheter also improved the CPR significantly compared to the Frydman catheter, and this is in agreement with other

studies reporting that the choice of catheter affects the CPR (10,11).

There were four ectopic pregnancies in our study (0.5%). This incidence is low compared to other studies (24,25). This may be due to our practice of routine trial transfer prior to initiation of treatment and/or the fact that all ETs were carried out by the same provider. It may also reflect the relative choice of patients as we have studied more ICSI patients (relative to IVF) with healthier pelvises. We have found that the incidence of ectopic pregnancies was not related to a particular catheter, to the difficulty of the procedure, or to presence of blood during ET. Other studies have reported a relationship between the incidence of ectopic pregnancies and the difficulty of the procedure (26–29). This discrepancy may be due to the rare nature of this complication of assisted conception.

In conclusion, we have found that a gentle and atraumatic technique and the use of the soft after-loading catheters significantly increase the CPR and IR for patients treated with IVF and ICSI. However, more randomized controlled studies are needed to confirm these findings.

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