Assisted reproduction outcomes after embryo transfers requiring a malleable stylet

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Abstract

Objective This retrospective case–control study was undertaken to assess the impact of a malleable stylet application on embryo transfer (ET) outcome.

Methods The Wallace malleable stylet was used when the soft inner catheter could not negotiate the internal os during ultrasound-guided ET. Patients having an age ≤ 37 years, with three embryos replaced were included in the analysis. Implantation rate, clinical pregnancy rate and live birth rate were the main outcome measures.

Results The Wallace malleable stylet was applied in 164 (29.6%) of the 553 ETs evaluated. Implantation rate, clinical pregnancy rate and live birth rate showed a 5.6% (19.4% vs. 13.8%), a 10.8% (41.9% vs. 31.1%) and a 9.9% (37.3% vs. 27.4%) absolute difference, respectively, in favor of ETs not requiring the stylet. These differences were statistically significant ($P < 0.05$).

Conclusions ETs requiring stylet are associated with lower implantation, clinical pregnancy and live birth rates.

Keywords Embryo transfer · Difficult transfer · Stylet · Assisted reproduction outcome

Introduction

Embryo transfer is recognized as a crucial phase in assisted reproduction technologies (ART), with the last decade having witnessed significant efforts in developments of techniques and instrumentation for ET. There is evidence suggesting that measures aimed at avoiding “difficult” transfers, including the use of soft catheters and ultrasound guidance, are important determinants for successful ET [1]. As a supportive example for this assertion, a study examining the outcome of 4,807 ETs found that easy or intermediate transfers yielded a 1.7 higher pregnancy rate in comparison to difficult transfers [2]. Despite improvements, ET is regarded as the most inefficient and vulnerable step in ART programs [3, 4]. Indeed, if the majority of ART women reach the ET stage, only approximately one third of these transfers results in a clinical pregnancy [5]. One of the most problematic step in the ET procedure is represented by the insertion of the catheter through the internal cervical os [1, 3]. Approaches used to overcome this problem and to facilitate ET include the use of firmer catheters or stylets [1, 3].

The Wallace malleable stylet has been designed to provide rigidity when using a Wallace Embryo Replacement Catheter and passage through the cervix is impeded or difficult (Wallace-Instruction for use). The inner catheter of the Edward Wallace fits the sheath of the stylet, permitting the insertion of the soft component of the Edward-Wallace catheter and thus allowing the embryos to remain in the same inner catheter [6].

There is limited scientific literature concerning the impact of Wallace malleable stylet application on ET outcome [6]. To improve upon this topic, the reproductive

Capsule Embryo transfer represents a crucial step in assisted reproduction procedures. We found that difficult embryo transfers requiring the use of a malleable stylet result in a poorer ART outcome

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outcome of patients undergoing to ET with an Edwards-Wallace embryo replacement catheter was retrospectively compared with that of patients needing the use of Wallace malleable stylet.

Materials and methods

Catheter and stylet

The Edwards-Wallace catheter 1816 N (23 cm) (Smiths Medical International Ltd, Hythe, Kent, U.K.) consists of a flexible and opening inner catheter and a detachable outer sheath that is attached to the inner catheter by a Luer compatible adapter. The inner catheter is 16 g with a lumen that is uniform throughout its length, with a series of 1 cm graduations at the proximal end. The inner catheter protrudes from the outer sheath by 5 cm and the outer sheath has a series of 1 cm graduations at the distal end (Wallace-Instruction for use). The Wallace malleable stylet 1816NST (Smiths Medical International Ltd, Hythe, Kent, U.K.), consists of a malleable inner stylet and a detachable outer sheath. With the hubs locked together the inner malleable stylet forms a smooth radius at the tip of the outer sheath (Wallace-Instruction for use).

Ovarian stimulation and in vitro fertilization

GnRH agonist or antagonist were used to prevent premature LH surges. Ovarian stimulation was accomplished using recombinant FSH (Puregon; Merk Sharpe and Dome, Italy; Gonal F, Merk Serono, Italy) or HMG (Meropur, Ferring, Italy) at daily doses ranging from 75 IU to 375 IU SC depending on individual patient response. When at least three follicles had reached a diameter of 18 mm, 5000–10000 IU of human chorionic gonadotrophin (hCG, Pregnyl, Merk Sharpe and Dome, Italy) were administered, and 36 h later transvaginal oocyte retrieval was performed under general anesthesia and ultrasound control. The collected oocytes were denuded and their nuclear maturation stage determined. In agreement with the Italian legislation on ART, a maximum number of three oocytes were fertilized, with all the resulting embryos being replaced during a single ET. Intracytoplasmic sperm injection (ICSI) was the mode of fertilization in all cases. The embryos were classified according to the following morphological criteria. Type A or excellent embryos are defined as embryos in which all blastomeres have an equal or non-equal size without fragmentation. The type B or good embryos have blastomeres of equal or non-equal size, with <20% of the volume of the embryo with anucleate fragments. The type C or fair embryos have anucleate fragments in 20–50% of the volume of

the embryo [7]. The luteal phase was supported by administration of 600 mg/day of micronized progesterone (Progeffik, Effik Italia, Italy).

Embryo transfer

The study retrospectively analyzed the outcome of fresh, non-donor ETs performed by a single provider between January 2005 and December-2010 in women having an age ≤ 37 years, and receiving three embryos. The Edwards-Wallace catheter is the exclusive ET catheter used in the clinic. Patients did not undergo mock transfer before their ET procedure, that was performed 48–72 h after ovum pick-up. Women were requested to have a full bladder to facilitate transabdominal ultrasonographic visualization of the cervical canal and uterine cavity. With the patient placed in dorsal lithotomy position, a speculum was positioned in the vagina to visualize the cervix, that was cleaned with sterile saline solution. Tenaculum was not used in any of the cases. Cervical mucus was gently removed with aid of a sterile cotton ball soaked in saline and using a sterile syringe. An Edward-Wallace catheter attached to a 1-ml sterile tuberculin syringe and containing the embryos was then inserted through the cervical canal and advanced into the mid-uterine cavity, taking care to avoid touching the uterine fundus. The embryos were then gently expelled and the catheter was slowly withdrawn with a rotating motion. The catheter was loaded with the three embryos taken up in a volume of approximately 10–20 µl of culture medium. Fluid inside the inner catheter was released and examined for retained embryos by removing the syringe from the catheter. Any retained embryos were immediately retransferred using a new catheter. When the Edwards Wallace catheter failed to negotiate the cervical canal, the transfer was accomplished with the aid of a Wallace malleable stylet. It was modulated and inserted through the cervical canal until the bulb tip of the catheter was located just past the internal os. The stylet was then removed living in place the outer sheath that served to insert the inner catheter containing the embryos and to perform ET.

Outcome measures and statistical analysis

The primary outcomes were the rates of implantation, clinical pregnancies and live births. Pregnancy test was performed by dosing βhCG serum blood, 14 days after ET. Clinical pregnancy was defined as the presence of a gestational sac with fetal hearth activity visible on ultrasound scan at seven weeks gestation. Ectopic pregnancies were not counted as gestational sac or clinical pregnancies. Live birth was defined as delivery of a living baby after at least 28 weeks of gestation. Statistical analysis was performed using the chi-squared test for proportions and Student's *t* test

Table 1 Main demographic and baseline characteristics of patients undergoing ET with stylet and without stylet

	With stylet (n=164)	Without stylet (n=389)	P value
Mean age in years	33.50±3.09	33.23±2.99	NS
Mean body mass index (kg/m ²)	22.17±3.51	22.59±4.04	NS
Basal FSH (IU/L)	7.64±2.99	7.44±3.00	NS
Etiology of infertility [n (%)]			
Male	54 (32.9%)	117 (30.1%)	NS
Female	63 (38.4%)	159 (40.9%)	NS
• Ovulation factor	10/63 (15.9%)	36/159 (22.6%)	NS
• Fallopian	36/63 (57.1%)	70/159 (44.0%)	NS
• Uterine	1/63 (1.6%)	10/159 (6.3%)	NS
• Endometriosis	16/63 (25.4%)	43/159 (27.0%)	NS
More than one diagnosis	18 (11.0%)	46 (11.8%)	NS
Unexplained	29 (17.7%)	67 (17.2%)	NS
Number of IVF attempts	1.54±0.92	1.61±1.09	NS
GnRH antagonist protocol	25/164 (15.2%)	73/389 (17.7%)	NS

Values are expressed as means ± SD or n (%)

for continuous variables. Values of $P<0.05$ were considered significant.

Results

The Wallace malleable stylet was applied in 164 (29.6%) of the 553 ETs evaluated. A summary of demographic and baseline characteristics of the two study groups is given in Table 1. There were no statistically significant differences between the two study groups with respect to the mean age, causes of infertility, basal FSH and body mass index (BMI).

Table 2 illustrates cycle outcomes of the two study groups. The majority of ET were performed 72 h. after ovum

pick up, namely in 64.6% of the stylet group and 58.4% of the without stylet group. Comparable results were observed in terms of mean number of retrieved oocytes, quality of the transferred embryos, implantation rate, pregnancy loss, ectopic pregnancy, biochemical pregnancies and multiple pregnancy rate. On the other hand, implantation rate, clinical pregnancy rate and live birth rate showed a 5.6% (19.4% vs. 13.8%), a 10.8% (41.9% vs. 31.1%) and a 9.9% (37.3% vs. 27.4%) absolute difference, respectively, in favor of ETs not requiring the stylet. These differences were statistically significant ($P<0.05$). The timing of ET did not apparently impact on cycle outcomes, being clinical pregnancy rates of the two study groups comparable after ET at 48 h or 72 h. The only other parameter that differed statistically between

Table 2 Comparison of cycle outcomes between patient undergoing ET with stylet or without stylet

	With stylet (n=164)	Without stylet (n=389)	P value
Retrieved oocytes (n)	9.11±4.62	8.97±5.34	NS
Metaphase II oocytes (n)	8.05±4.45	6.88±2.59	0.000
Biochemical pregnancy only	11/164 (6.7%)	15/389 (3.8%)	NS
Implantation rate	68/492 (13.8%)	226/1167 (19.4%)	0.009
Pregnancy loss	6/51 (11.8%)	18/163 (11.0%)	NS
Ectopic pregnancy	3/164 (1.8%)	10/389 (2.6%)	NS
Clinical pregnancy	51/164 (31.1%)	163/389 (41.9%)	0.022
Grade A and B embryos	391/492 (79.5%)	878/1167 (75.2%)	NS
Number of ET at 48 h	58/164 (35.4%)	162/389 (41.6%)	NS
Number of ET at 72 h	106/164 (64.6%)	227/389 (58.4%)	NS
Clinical pregnancy/ET at 48 h	19/58 (32.8%)	69/162 (42.6%)	NS
Clinical pregnancy/ET at 72 h	32/106 (30.2%)	94/227 (41.4%)	NS
Twin pregnancy	11/51 (21.6%)	47/163 (28.8%)	NS
Triplets	3/51 (5.9%)	8/163 (4.9%)	NS
Live birth rate	45/164 (27.4%)	145/389 (37.3%)	0.033

Values are expressed as means ± SD or (%)

Italian legislation on ART allows fertilization of a maximum number of three oocytes, with all the fertilized oocytes being replaced and during a single ET. Fertilization rate was 100% considered that only women having three embryo transferred were included in the analysis

In all instances eggs were fertilized by intracytoplasmic sperm injection (ICSI)

the two study groups was the number of metaphase II oocytes, that was higher in the group needing the stylet.

Discussion

Results of this study indicate that application of the Wallace malleable stylet to facilitate ET is associated with a lower implantation, clinical pregnancy and live birth rates in comparison to uncomplicated ETs carried out with Wallace Embryo Replacement Catheter. Our results are in contrast with findings of Nielsen et al. [7] who compared, in a retrospective case control study, the cycle outcome of 205 stylet and 410 uncomplicated ETs, and reported no differences in implantation and live birth rates between the two study groups. Looking at possible explanations for contradiction in findings, we noted that in the Nielsen et al. [6] study a tenaculum was routinely applied during ET to expose and straighten the cervical canal. In contrast, the tenaculum was never applied in our case series. Traction of the cervix with a tenaculum is applied to favor the insertion of the catheter, and may have thus reduced the need of stylet ETs. Indeed in the Nielsen et al. study [6] the percentage of unsuccessful ET needing the aid of the malleable stylet was there time lower (10.2%) than in our study (29.6%). In a prospective randomized comparison of the Wallace and the Cook Echo-Tip catheter, where a tenaculum was never used in any of the cases, the percentage of patients needing the use of a stylet was about 26% [8] and thus comparable to the percentage recorded in this study. More to the point, difficulties in accessing the endometrial cavity have been encountered in 21.7% [9], and in 28.2% [10] of ETs using soft coaxial transfer catheter.

It has been previously shown that the individual provider can strongly influence pregnancy rates after ET [11]. The fact that in this study all the ET were performed by a single provider rules out the so called “physician factor”. Other strengths of the study are that all patients received the same number of embryos and that the same in vitro fertilization technique (ICSI) was applied in all instances. All the ICSI were performed by a single embryologist.

This study indicates that stylet ETs are associated to poorer ART outcomes. The study also suggests that methodologies aimed at preventing the use of stylets, including trial transfers and cervical dilatation, should be considered. Our results are in line with the well supported concept that difficult ETs result in reduced chances of successful implantation [1, 3]. Determinants of reduced chances of implantation during difficult or traumatic ETs have not completely

understood. Stimulation of uterine contraction secondary to cervical manipulation, disposition of the embryos in a sub-optimal uterine site [1], or disruption of the endometrium by the catheter have been implicated [12, 13].

Conflict of interests The Authors declare no potential conflict of interest.

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