The impact of the depth of embryo replacement on IVF outcome

Arianna Pacchiarotti · Mohamed A. Mohamed · Giulietta Micara ·
Daniela Tranquilli · Antonella Linari · Salomè M. B. Espinola · Cesare Aragona

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Abstract *Purpose:* To evaluate the effect of the depth of embryo transfer replacement on clinical pregnancy rate.

Methods: Data from a total of 104 consecutive embryo transfers performed on 104 women aged 26–37 years were prospectively collected for this study. All patients underwent a standard down regulation protocol for ovarian stimulation. Oocytes retrieval were performed at 36 h after hCG administration. Embryo transfer took place at 48 h after insemination. The patients were matched in two groups according to the distance between the tip of the catheter and the uterine fundus at transfer (group A > 10 < 15 mm and group B ≤ 10 mm). The same method of loading embryos into the embryo transfer catheter was used.

Results: Clinical pregnancy rates varied significantly ($p \leq 0.05$) between the two groups: 27.7% in group A and 14% in group B. The number and quality of embryos transferred did not differ between the groups.

Conclusions: The results suggest that the depth of embryo replacement may be an important variable in embryo transfer technique.

Keywords Embryo transfer · Depth of replacement · Pregnancy rate · Implantation rate

Introduction

Embryo transfer is maybe the most important, final step in IVF (in vitro fertilization) and intracytoplasmic sperm injection (ICSI) treatment. The embryo transfer can be performed in three ways: (1) blind (clinical touch); (2) based on information on the length of the uterus (obtained by previous ultrasonographic measurement or mock transfer); (3) guided by abdominal ultrasonography. It is advantageous to place the embryos into the uterus in as atraumatic a manner as possible [1–3]. Uterine contractions [4, 5], presence of blood on the catheter [6, 7], bacterial contamination of the catheter [8], difficulty of transfer [9], and the type of catheter [10–13], all influence the success rate of the IVF/ICSI treatment. The embryos should be placed in an area within the uterus most likely to afford implantation. Some [14–17] have suggested that ultrasound guidance of the embryo transfer procedure may be of benefit because the tip of the embryo transfer catheter can be visualized to ensure that the embryos are placed in the proper location. Some [18] show that pregnancy and implantation rates can be dramatically affected by the physician performing the embryo transfer.

A high success rate has been reported with a technique of transabdominal ultrasound guidance in the presence of a full bladder [19]. Most IVF teams consider not touching the endometrium and the uterine fundus with replacement of the embryos in the lumen of the endometrial cavity the most important factors for successful embryo transfer [20, 21]. It has been traditionally accepted that the embryo should be placed ~ 10 mm below the fundal endometrial surface [22], some authors have suggested that placing embryos rather lower in the uterine cavity may improve pregnancy rate [23]. The aim of our study was to assess the importance of the depth of embryo replacement into the uterine cavity measured by ultrasound and its influence on implantation rate after IVF.

A. Pacchiarotti (✉) · M. A. Mohamed · G. Micara ·
D. Tranquilli · A. Linari · S. M. B. Espinola · C. Aragona
Dipartimento di Scienze Ginecologiche, Perinatologia e
Puericultura Policlinico Umberto I° Università di Roma, “La
Sapienza” Viale Regina Elena,
Rome, Italy
e-mail: arypac@aliceposta.it

Praxi Pro Vita Centro di Fertilità,
Via Magnagrecia 117, Rome, Italy

The study was approved by Institutional Ethical Committee of the Department of Obstetrics and Gynaecology of the University of Rome.

Method

Between February and May 2006, 108 consecutive patients underwent ultrasound-guided embryo transfer. Transfers of cryopreserved embryos or embryos resulted from oocytes donation were not included. Randomization was made between aged 26–37 years patients with the following criteria: (1) main causes of infertility attributable to tubal, idiopathic, or male factors, (2) serum levels of FSH and LH on day 3 of the ovarian cycle is less than 12 IU/L, (3) regular menstrual cycle, and (4) normal uterine cavity. A total of 108 patients who met the above-mentioned criteria were enrolled in this study. To identify patients with potentially difficult ET, all patients underwent a trial embryo transfer in a cycle preceding the IVF treatment in order to measure the uterine cavity depth and direction of the cervix and the uterus. These patients were randomly assigned to two study groups according to the distance between the tip of the catheter and the uterine fundus at the time of embryo deposition in the cavity: group A: $> 10 < 15$ mm; group B ≤ 10 mm. Our IVF cycle management consisted of down regulation with gonadotrophin-releasing analogue s.c. daily (Decapeptyl 0.1, Ipsen) from day 21 of the pre-treatment cycle. Recombinant FSH (Gonal F Serono, Rome, Italy) was started on day 3 of the treatment cycle until HCG day. HCG 10000 UI (Gonasi HP, Amsa, Rome, Italy) was given i.m. when 50% of follicles reached 20 mm of diameter. Transvaginal ultrasound-guided oocytes retrieval was done 36 h after HCG administration. The luteal phase was supplemented with i.m. progesterone 100 mg daily (Prontogest, Amsa, Rome, Italy). Ultrasound guided embryo transfer was performed at day 2 as follows. Patients were recommended to come into our unit with a relatively full bladder; the Edwards- Wallace embryo replacement catheter connected to an insuline syringe was used for all embryo transfer. This is a soft silicon catheter that possesses a stiffer outer sheath that stabilizes the softer inner cannula which carries the embryos. Ultrasound monitoring was performed using transabdominal imaging with a 3.5 MHz linear array probe. The bladder was filled with the intention to both enhance imaging of the uterus and endometrial lining and to straighten any uterine flexion that may be present. Scout imaging of the pelvis was then carried out transvesically and the uterus and fundal region of the endometrium brought into focus. A bivalved speculum was then inserted into the vagina and the vaginal vault carefully swabbed with sterile saline solution with Bergamon α 5% to clear the area of any mucus or debris. The coaxial system, loaded with the embryos in the transfer catheter was then brought into the field.

Both transfer and guide catheter was advanced as a unit. The guide catheter should serve to stabilize the softer and more flexible transfer catheter. The transfer catheter should be kept well within the rigid guide catheter to reduce any unintended movement of the transfer catheter tip. Care has been taken not to advance the guide catheter into the depth of the endometrial cavity as its rigid structure may disrupt the endometrial lining. The guide catheter was advanced just beyond the external os. Under direct transabdominal ultrasound guidance, the transfer catheter was then advanced through the endocervical canal into the lower uterine segment. Immediate identification of the catheter tip is essential to minimize motion of the catheter and avoid any impact on the endometrium. The transfer catheter may then be advanced to a defined distance from the uterine fundus, > 10 mm < 15 and ≤ 10 mm respectively in A and B group. This measurement may be easily verified by using the calipers prior to injection of the embryos. After transfer, the catheter was held in place for 45 to 60 seconds to permit the embryos to settle away from the catheter tip. The transfer and guide catheters were then slowly withdrawn as a unit and inspected for any retained embryos and to detect bleeding. Transfer was performed by the same provider in all patients included in the present study. The same transfer technique was maintained with all patients.

Increasing serum concentration of beta HCG and the presence of intrauterine gestational sac demonstrated pregnancy.

Statistical analysis were performed using χ^2 -test to compare qualitative variables and Student's *t*-test to compare quantitative variables. The significancy level was set at $p = 0.05$.

Results

Four patients were cancelled: three woman in group A and one women group B, because the excessive ovarian response leading to high risk for OHSS (5.3 and 2% of patients, respectively). This difference was not statistically significant ($P = 0.91$). Of the 108 studied patients, 104 underwent oocyte retrieval, 54 patients in group A and 50 in group B.

There were not significantly differences between the two groups in term of embryo transfer characteristics (Table 1).

Both groups proved to be comparable regarding the main demographic characteristics (Table 2), as well as the main cycles parameters: these variables showed infact statistically non significant differences.

Our results show that there was not significantly differences between two groups in term of IU of FSH administred (3780 ± 543 in group A vs. 3633 ± 868 in group B), estradiol levels on the day of HCG day (1897 ± 761 in group A vs. 1902 ± 752 in group B), mean of oocytes retrieval and embryos transferred (ET) (8.1 ± 2.4 vs. 7.2 ± 2.1 and

Table 1 Embryo transfer characteristics

	Group A (<i>n</i> = 54)	Group B (<i>n</i> = 50)	<i>P</i> value
Endometrial thickness on the day of ET $\pm$ sd	11.2 $\pm$ 2.08	12.1 $\pm$ 2.2	.681
Full bladder	51.85% (27)	48.% (24)	.083
Ease of transfer % (<i>n</i>)	72.2% (39)	68% (34)	.361
Use of tenaculum % (<i>n</i>)	5.6% (3)	8% (4)	.299
Bleeding	1.8% (1)	2% (1)	.739

Table 2 Demographic characteristics

	Group A	Group B	<i>P</i> -value
<i>N</i>	54	50	NS
Mean Age $\pm$ sd	34.1 $\pm$ 2.1	32.6 $\pm$ 1.7	NS
Duration of infertility $\pm$ sd	4.31 $\pm$ 1.03	4.47 $\pm$ 1.05	NS
Primary infertility (<i>n</i>)	62.9% (34)	62% (31)	NS
Tubal factor (<i>n</i>)	51.8% (28)	52% (26)	NS
Male factor (<i>n</i>)	40.7% (22)	42% 21	NS
Idiopathic infertility (<i>n</i>)	7.4% 4	6% 3	NS
Mean cycles $\pm$ sd	2.8 $\pm$ 0.5	3.1 $\pm$ 0.7	NS

Table 3 Clinical Outcome by the different depth of replacement

	Group A	Group B	<i>P</i> -value
<i>N</i>	54	50	.636
IU FSH $\pm$ sd	3780 $\pm$ 543	3633 $\pm$ 868	.899
Estradiol on the day of HCG $\pm$ sd	1897 $\pm$ 761	1902 $\pm$ 752	.766
Mean of oocytes retrieval $\pm$ sd	8.1 $\pm$ 2.4	7.2 $\pm$ 2.1	.542
Mean of ET $\pm$ sd	2.1 $\pm$ 1.2	1.8 $\pm$ 1.6	.838
I grade ET	50.2%	53.7%	.752
II grade ET	38.8%	34.2%	.443
III grade ET	11%	12.1%	.388
PR	27.7%	14%	.009
IR	15.3%	7.1%	.027
GEU	1.8%	4%	.321
Multiple pregnancy	0%	0%	.661
Abortion rate	1.8%	2%	.553

2.1 $\pm$ 1.2 vs. 1.8 $\pm$ 1.6 respectively in group A and B), and grade of ET. (I grade 50.2% vs. 53.7%, II grade 38.8 vs. 34.2% and 11% vs. 12.1% respectively in group A and B) It was found a statistically significantly difference in pregnancy rate (PR) that results 27.7% in A group vs. 14% in group B ($P = 0.009$) and in implantation rate ($P = 0.027$) (15.3% vs. 7.1% respectively in group A and B). There was no difference in abortion rate and ectopic pregnancy rate between the two groups studied (Table 3).

Discussions

Our data seems to show a significant increase in pregnancy rate and implantation rate in A group, where there was a distance $> 10 < 15$ mm between the tip of the catheter and uterine fundus. Since the earliest descriptions of in vitro fertilization (IVF), clinical and laboratory aspects of

this procedure were considered the driving forces behind improved pregnancy rates and embryo transfer techniques were of secondary importance. Techniques, equipment and materials for embryo transfer catheters remained relatively unchanged until recently. Earlier techniques used single lumen, relatively rigid catheters passed into the uterine cavity under clinical touch only. Though the production of quality embryos remains the goal, there is an increasing appreciation of the embryo transfer technique as a variable that can significantly impact pregnancy rates. Several factors have emerged that can impact success of any embryo transfer including the vaginal flora, experience of the clinician(s) performing the transfer [18], and volume of transfer media [3]. Evidence suggests that blind embryo transfer may result in placement of the embryos outside the uterine cavity in as much as 25–30% of cases [24]. The two most significant changes in embryo transfer techniques are improvements in catheter quality and guidance techniques enabling accurate

and atraumatic embryo replacement. There is a trend in deviating from rigid, single lumen catheters—towards the use and continued development of coaxial soft tipped catheter systems; and a trend away from replacement of the embryos under tactile guidance only—toward ultrasound guidance for embryo transfers. These improvements have resulted in a more efficient transfer technique and improved pregnancy rates, though this mechanism is not clearly understood.

About the influence of the depth of embryos replacement into the uterine cavity we found different postulation. Most common, from the beginning of IVF, embryos have been placed 1 cm below the top of the cavity. Some authors have suggested that PR may be higher when embryos are placed rather lower in the uterin cavity [19] and others suggested that replacing embryos within 1 cm of the uterine endometrial fundus may be associated with a high tubal PR [25, 26], others have reported that PR it is a bit higher when embryos are placed high in the uterus and have supposed that low fundal implantation may be partly responsible for the higher rate of spontaneous abortion [27, 28].

What we want to show in our study is that, although baseline patient clinical characteristics, treatment cycles characteristics, ovarian response and any variable of transfer (difficulty of transfer, bleeding, use of tenaculum) were all similar in the two groups studied, we found a statistically significant difference in PR and IR between the two groups. We avoided any impact of the physician factor on implantation rate because all transfer were carried out by the same provider. In addition we didn't find a statistically significant difference in abortion rate between the two groups studied. Ectopic pregnancies observed in our study occurred approximately with the same percentage in group A and B. Nevertheless our sample size was too small to avoid the occurrence of type II error.

In conclusion, may be the depth of the embryo replacement should be important to improve success of implantation rate, though more studies are warrented to confirm our results.

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