Effect of the afterloaded external guidance embryo transfer technique on pregnancy rates in single embryo transfer cycles

Dış kateter yönlendirmesi ile embriyo transfer tekniğinin tek embriyo transferi sikluslarında gebelik oranlarına etkisi

Nafiye Yılmaz[1], Ayla Sargın Oruç[1], Tugba Zeyrek[2], Ümit Görkem[1], Hasan Ali İnal[1], Yaprak Engin-Üstün[1], Cavidan Gülerman[1]

[1]IVF Department, Zekai Tahir Burak Women's Health Research and Education Hospital, Ankara, Turkey

[2]Department of Embryology, Zekai Tahir Burak Women's Health Research and Education Hospital, Ankara, Turkey

Abstract

Objective: To investigate effect of the afterloaded external guidance embryo transfer technique on pregnancy rates in single embryo transfer intracytoplasmic sperm injection (ICSI) cycles.

Material and Methods: This retrospective study was performed at the Dr. Zekai Tahir Burak Women's Health Research and Education Hospital. Three hundred and thirteen women who underwent ICSI were included in the study. Subjects were categorized according to the embryo transfer technique; Group 1 (n: 232): easy transfer with a soft catheter, Group 2 (n: 45): after external guidance transfer, and Group 3 (n: 36): difficult transfer with a stylet. Basal parameters, clinical and laboratory IVF outcomes and pregnancy rates were studied.

Results: Infertility etiology, basal follicle stimulating hormone (FSH) levels, antral follicle count, duration of stimulation, total dose of gonadotropin, peak estradiol levels, endometrial thickness, oocyte number, 2 PN, and fertilization rate were similar between the three groups (p>0.05). Despite the decreased pregnancy rate in Group 3, there were no differences in clinical pregnancy rates among the groups (p=0.204).

Conclusion: Embryo transfer is one of the critical steps in assisted reproduction procedures. Using the afterloaded external guidance embryo transfer technique did not improve pregnancy rates.

(J Turkish-German Gynecol Assoc 2013; 14: 153-6)

Key words: Embryo transfer technique, pregnancy rates, IVF

Received: 27 June, 2013 **Accepted:** 19 July, 2013

Özet

Amaç: Dış kateter yönlendirmesi ile embriyo transferinin tek embriyo transferi yapılan intrasitoplazmik sperm injeksiyonu (ICSI) siklusların-da gebelik oranlarına etkisinin araştırılmasıdır.

Gereç ve Yöntemler: Zekai Tahir Burak Kadın Sağlığı Eğitim ve Araştırma hastanesinde gerçekleştirilen retrospektif çalışmaya ICSI uygulaması yapılan 313 hasta dahil edilmiştir. Hastalar embriyo transfer tekniğine gore gruplara ayrıldı; Grup 1 (n: 232): yumuşak kateter ile kolay transfer, Grup 2 (n: 45): iç-dış kateter uygulaması ile transfer, Grup 3 (n: 36): Sert kateter ile gerçekleştirilen zor transfer. Bazal parametreler, klinik ve laboratuar ICSI sonuçları ve gebelik oranları araştırıldı.

Bulgular: Üç grubun, infertilite sebepleri, bazal folikül stimule edici hormon (FSH) düzeyleri, antral folikül sayıları, stimülasyon süresi, total gonadotropin dozu, pik estradiol düzeyleri, endometrial kalınlık, toplanan yumurta sayısı, 2 PN, fertilizasyon oranları benzerdi (p>0.05). Grup 3'te gebelik oranları daha düşük olsa da klinik gebelik oranlarında fark izlenmedi (p: 0.204).

Sonuç: Embriyo transferi yardımcı üreme tekniklerinde kritik bir adımı oluşturmaktadır. Dış kateter yönlendirmesi ile embriyo transferi uygulaması gebelik oranlarını arttırmamıştır.

(J Turkish-German Gynecol Assoc 2013; 14: 153-6)

Anahtar kelimeler: Embryo transfer tekniği, gebelik oranları, IVF

Geliş Tarihi: 27 Haziran 2013 **Kabul Tarihi:** 19 Temmuz 2013

Introduction

The pregnancy rate after embryo transfer (ET) depends on clinical and embryonic characteristics. Apart from embryo quality, endometrial receptivity and the age of the patient, pregnancy rate after ET seems to be mostly dependent on the ET technique. Cohen reported that 'bad embryo transfer' is responsible for failed implantation in 30% of cases (1). Although the importance of the ET has been suggested by studies comparing different operators or different ET catheters and techniques, the relationship between the ET technique, catheter type, operator and the clinical pregnancy rate is still controversial (2-7).

Mansour reported the use of a mock ET before starting an IVF cycle in 1990 (2). A mock ET allows the physician to choose the appropriate transfer catheter and anticipate potential problems during ET. However, a mock transfer remote from the actual ET is done under different circumstances and may not be reflective of the actual conditions encountered on the day of ET (3). Sharif et al. (3) proposed to circumvent this problem by performing a mock ET immediately before the actual ET. To avoid additional trauma by the passage of two

Address for Correspondence: *Ayla Sargın Oruç, IVF Department, Zekai Tahir Burak Women's Health Research and Education Hospital, Ankara, Turkey. Phone: +90 312 285 47 24 e.mail: aylasrgn@yahoo.com*

doi:10.5152/jtgga.2013.49225

separate catheters, Neithardt began transferring embryos by an afterload technique (afterloaded external guidance), in which an empty catheter is placed at, or just past, the internal cervical os. The inner sheath is withdrawn, and a second inner sheath containing the embryos is passed. This gives the provider the benefit of an immediate mock transfer while minimizing manipulation of embryos and possibly reducing trauma to the endometrium (6).

We performed a retrospective analysis of 313 single ET ICSI cycles to determine the impact of the transfer technique on pregnancy rates.

Material and Methods

From March 2010 to February 2012, at the Dr. Zekai Tahir Burak Women's Health Research and Education Hospital, IVF Department, 313 consecutive single ET ICSI cycles were retrospectively reviewed. This study was approved by the ethics committee and institutional review board of Dr. Zekai Tahir Burak Women's Health Research and Education Hospital. Written informed consent was obtained from all volunteers. A total of 313 otherwise healthy women who complained of primary infertility were eligible. Only fresh cycles were included.

Pituitary down-regulation was achieved and maintained using the long protocol luteal phase administration of a GnRH agonist. The GnRH agonist leuprolide acetate (Lucrin daily; Abbott Cedex, Istanbul, Turkey) was initiated on day 21 of the preceding luteal phase (0.5 mg/d SC) until menses and dropped to 0.25 mg/d until triggering ovulation. Recombinant (rec) FSH (Puregon; Organon, Oss, the Netherlands; or Gonal F; Serono, Istanbul, Turkey) was used for ovulation stimulation. The initial gonadotropin dose used for ovarian stimulation was individualized according to the patient's age, baseline serum FSH concentrations on day 3, body mass index, and previous response to ovarian stimulation. The starting regimen was fixed for the first 3 days (100-225 IU rec FSH/day), and thereafter the dose of gonadotropin was adjusted according to the individual ovarian response. Serum estradiol concentrations and transvaginal ultrasonography were measured routinely every 2-3 days thereafter. Ovulation was triggered by administration of rec HCG (Ovitrelle, 250 μgr, Serono, Istanbul, Turkey) when at least two follicles reached 18 mm in diameter. Oocytes were retrieved at 36 hours after HCG injection and subjected to intracytoplasmic sperm injection regardless of infertility origin. ET was done on day 3.

Embryo transfer technique

All ETs were performed with a full bladder under ultrasound guidance (Aloka SSD-1000, Germany) using a catheter (Rocket Genesis embryo transfer catheter system R57630-00-23, R57591-00-23). The difficulty of the ET was determined according to the opinions of two physicians with the same practice and experience with the transfer technique. The scored difficulty of transfers generated by the two physicians was the same for all the transfers. The ETs were scored as easy transfer with a soft catheter, moderate transfer with external guidance, or difficult transfer with a stylet.

Direct Embryo Transfer

The patient was placed in the lithotomy position. A sterile bivalve speculum was placed in the patient's vagina and the cervix was exposed. Excess mucus and debris were cleared from the ectocervix using sterile cotton swabs dampened with phosphate-buffered saline. The embryos were loaded into the transfer catheter by the embryologist as described elsewhere, and the catheter was passed to the transfer physician. The embryos were then deposited approximately 1.0 cm from the uterine fundus under ultrasound guidance. After approximately 5 seconds, the catheter was gently removed. The embryologist immediately flushed the catheter with media to check for retained embryos, blood, or mucus. Patients remained supine for 10 minutes after the procedure.

Difficult transfers were managed first with the use of external guidance with introduction of the catheter through an advanced sheath rather than with a stylet. We excluded cycles in which a teneculum was used.

Afterloaded External Guidance Embryo Transfer

An empty catheter was passed to the level of the lower uterine segment under ultrasound guidance to a point where the inner catheter entered the endometrial cavity. The inner sheath was slowly removed, leaving the outer sheath just beyond the internal os. A second inner sheath was loaded by an embryologist who then assisted the transfer physician in threading the inner sheath into the catheter. The inner catheter was slowly advanced by the physician, and the embryos were deposited 1.0 cm from the fundus. After approximately 5 seconds, the catheter was gently removed. The embryologist immediately flushed the catheter with media to check for retained embryos, blood, or mucus. Patients remained supine for 10 minutes after the procedure.

Luteal phase support was routinely given as progesterone in the form of Crinone 8% gel (90 mg; Serono) daily for 14 days, until a pregnancy test was performed.

Subjects were categorized according to embryo transfer technique; Group 1 (n: 232): easy transfer with a soft catheter, Group 2 (n: 45): afterloaded external guidance transfer, Group 3 (n: 36): difficult transfer with a stylet. ETs with blood (n: 9) or mucus (n: 3) on the catheter tip were not included in the study. Data were collected for baseline (age, day 3 FSH level, antral follicle count) and stimulation parameters (duration of stimulation, total gonadotropin dose, number of follicles, peak estradiol levels, endometrial thickness), and cycle outcome (oocytes, mature oocytes, embryo transfer type and pregnancy rate). Clinical pregnancies were defined as those with fetal heart activity documented on ultrasound examination 4-5 weeks after embryo transfer.

Data analysis was performed by using SPSS for Windows, version 11.5 (SPSS, Inc., Chicago, IL, USA). We used one-way ANOVA, Kruskal Wallis, and Chi-square tests for the analysis. A P value less than 0.05 was considered statistically significant.

Results

Mean age, baseline FSH, estradiol levels, antral follicle count, duration of stimulation, gonadotropin dose, peak estradiol lev-

els, endometrial thickness, oocyte number, 2 PN, and fertilization rate were not different among the three groups (p>0.05). Despite the decreased pregnancy rate in Group 3, there were no differences in clinical pregnancy rates between the groups (p=0.204). All results are shown in Table 1.

Discussion

The ET technique is equally important as clinical and embryonic characteristics regarding the pregnancy rate in ART cycles. In the last decade, it has been demonstrated by many studies that differences in technique may affect pregnancy rates (2-7). There have been studies comparing the effect of different transfer catheters, different operators or transfer type on outcome. Although Burke reported that the difficult ET did not affect the clinical pregnancy rate, Abusheika found that a difficult embryo transfer technique negatively affects pregnancy rates (8, 9). On the other hand, a meta-analysis by Abou-Setta demonstrated that softer catheters are associated with higher clinical pregnancy rates than firmer catheters by overall comparison (10). Zhan Yao found that variation in pregnancy rates between embryo transfer catheters depends on variation between operators (11). Many investigators reported improved pregnancy rates when uterine contractions were minimized by the use of soft catheters, ultrasound guidance and fixed distance transfers (4, 6, 12). To avoid additional trauma by the passage of two separate catheters, Neithardt began transferring embryos by an afterload technique (afterloaded external guidance) (6). We performed a retrospective analysis of 313 single ET ICSI patients to determine whether there were differences in pregnancy rates based on the transfer technique using external guidance. In this study, we had a rather homogenous group of patients receiving only an agonist protocol with single ET cycles. All transfers were done on day 3. In our study, two senior operators performed all ETs, which in turn minimized the impact of operator skill and experience on the outcome. There were no differences in the baseline parameters like, day 3 FSH and E2, antral follicle count, duration of the stimulation, total dose of gonadotropin, number of oocytes retrieved and 2 PN, and fertilization rates. In our study, all transfers are performed under ultrasound guidance and at a fixed distance of 1 cm from the fundus which is the favored location according to recent studies (13). The results of the present study show that difficult transfers were associated with a lower clinical pregnancy rate (CPR) compared to easy transfers and afterloading, but this did not reach statistical significance. Difficult transfers were associated with the lowest CPR, but the number of cases in this group was relatively smaller than the others.

Neithardt et al. compared direct ET with afterloaded ET and reported an improved implantation rate (20.5% vs. 24.7%) and CPR (34.9% vs. 52.4%) with afterloading, although the difference was not statistically significant. They also found significantly more transfer catheters with mucus in the direct transfer group. Mucus on the transfer catheter has been proposed to adversely affect implantation either by contamination of the cavity or by causing retention or displacement of the embryos. The authors explained this by avoidance of passage of embryos through the initial inner sheath placed in the cervical canal, which they

Table 1. Clinical and laboratory parameters of the groups

	Easy (n:232)	External guidance (n:45)	Difficult transfer with a stylet (n:36)	p
Age (years)	29.7±4.5	29.3±4.1	28.6±4.1	0.339
Infertility etiology				0.126
Male	107 (46.1%)	24 (53.3%)	25 (69.4%)	
Tubal	12 (5.2%)	2 (4.4%)	1 (2.8%)	
Unexplained	113 (48.7%)	19 (42.2%)	10 (27.8%)	
Baseline FSH (IU/L)	6.1 (0.5-12.1)	6.6 (3.6-10.7)	5.7 (2.5-9.2)	0.088
Baseline E2 (pg/mL)	55 (4.3-176)	58 (23.2-153)	56.5 (14-126)	0.590
Antral follicle count (n)	11 (2-16)	10 (3-14)	12.5 (1-16)	0.825
Duration of stimulation (days)	10 (6-23)	10 (6-14)	10 (6-17)	0.933
Gonadotrophin dose (IU)	1650 (475-7700)	1900 (600-5850)	1687.5 (750-4400)	0.498
Peak E2 (pg/mL)	2215 (192-6867)	2379 (547-5963)	2490 (910-4988)	0.706
Endometrial thickness (mm)	10 (5-18)	10 (5-15)	11 (6-19)	0.160
Oocyte number	9 (1-32)	8 (1-28)	10 (3-28)	0.440
2PN	4 (1-15)	5 (1-12)	4 (1-17)	0.308
Fertilization rate (%)	60 (0.5-100)	50 (12.5-100)	50 (10-100)	0.130
Clinical pregnancy rate (%)	92 (39.7%)	15 (33.3%)	9 (25.0%)	0.204

Values are expressed as mean±standard deviation, median (interquartile range), n (%), p<0.05 is significant,
FSH: follicle stimulating hormone; E2: estradiol; PN: pronucleus

believe decreased mucus contamination of the catheter (6). Our study is different from Neithardt's study since we excluded all ETs with blood or mucus on the catheter tip to evaluate the impact of the technique rather than other confounding factors; furthermore, we included only single ET cycles. Our findings are consistent with their results with regard to similar CPRs in external guidance and easy transfer with a soft catheter ETs.

In another study, a cohort of 784 consecutive cycles with four different types of catheters were compared: a) a rigid preloading-type catheter b) a rigid afterloading type with an obturator and a soft inner catheter c) a ball-pointed rigid afterloading catheter with a soft inner catheter d) an afterloading type catheter with an obturator and an inner ultrasoft catheter. All ETs were performed by a single operator. The ultrasoft catheter was found to produce the highest pregnancy and implantation rates compared to the more rigid Frydman catheter. Negotiation of the cervix, using the volsellum, and the presence of the blood on the catheter wall or on the cervix did not affect the results. Changing the catheter or blood on the catheter tip significantly diminished the pregnancy and implantation rates (12). Sallam et al. (12) reported a low CPR with a rigid catheter compared to external guidance. In our study, despite the decreased pregnancy rates in the rigid catheter group, there were no differences in the clinical pregnancy rates between the groups. This may be attributable to the small size of our rigid catheter group. Recently, Spitzer et al. (7), reported lower CPR and live birth delivery rate (LBDR) in the external guidance ET group than the soft catheter ET group and the group in which the cervix was probed with a stylet (7). However, the groups of patients were not homogenous in this study and D5 transfers were done in a great majority of patients, which is not the case in most clinical settings. Furthermore, LBDR is mainly related to embryonic characteristics rather than the ET technique. Spitzer et al. estimated that external guidance may be related to a greater risk of transferring mucus and blood into the fundus, which is in contrast to the findings of Neithardt et al. We excluded ETs with blood or mucus on the catheter tip to minimize the negative effect on implantation.

In conclusion, we recommend an afterloaded external guidance ET technique when direct ET with a soft catheter is not available, especially in training centers like our institution. It is a simple procedure with similar CPR as direct ET with a soft catheter. Randomized controlled trials with a large number of patients are required to identify the impact of different ET techniques on ART outcomes.

Ethics Committee Approval: *Ethics committee approval was received for this study.*

Informed Consent: *Written informed consent was obtained from the participants of this study.*

Peer-review: *Externally peer-reviewed.*

Author contributions: *Concept - N.Y.; Design - N.Y.; Supervision - N.Y., A.S.O., Ü.G., H.A.İ., Y.E.Ü., C.G.; Resource - N.Y., A.S.O., Ü.G.; Materials - N.Y., A.S.O., T.Z., Ü.G., H.A.İ.; Data Collection&/or Processing - N.Y., A.S.O., T.Z., Ü.G., H.A.İ.; Analysis&/or Interpretation - N.Y., A.S.O., H.A.İ., Y.E.Ü., C.G.; Literature Search - N.Y., A.S.O.; Writing - N.Y., A.S.O., Y.E.Ü.; Critical Reviews - N.Y., A.S.O., Y.E.Ü., C.G.*

Conflict of Interest: *No conflict of interest was decleared by the authors.*

Financial Disclosure: *No financial disclosure was decleared by the authors.*

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