Effects of embryo transfer quality on pregnancy and live birth delivery rates

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Abstract

Background To analyze the effects of embryo transfer (ET) quality on clinical pregnancy (CPR) and live birth delivery rates (LBDR).

Methods In a retrospective study at a single, private infertility center between November 2005 and December 2009 one thousand fifty-five day-3 and day-5 ETs following IVF/ICSI/IMSI were evaluated. We analyzed the impact of an atraumatic ET with a soft catheter (ET 1), after external guidance (ET 2), after probing of the cervix with a stylet (ET 3), or after grasping the portio vaginalis with a tenaculum (ET 4) on CPR and LBDR.

Results The use of external guidance showed a significantly reduced LBDR as compared to an atraumatic ET (26.0% vs. 32.5%). The lowest CPR and LBDR were found in ET 4. The application of stylets in cases of difficult ETs was

superior to the use of external guidance. No differences in miscarriages between ET 1–4 were noted.

Conclusions Besides embryo culture and patient history, the quality of an ET might also have an important impact on pregnancy outcome. Techniques to ensure an atraumatic ET, such as mechanic uterine cavity length measurements, before starting treatment might help identify patients at risk for a difficult ET and lead to modified treatments, such as the primary use of a stylet. Limitation of study: retrospective analysis

Keywords Embryo transfer quality · Embryo transfer technique · Assisted reproduction · Clinical pregnancy rates · Live birth delivery rates

The authors have nothing to disclose.

Capsule The quality of embryo transfer (ET) might have an impact on pregnancy outcome. Techniques to ensure an atraumatic ET before starting an IVF cycle might help in optimizing the treatment of patients.

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Introduction

It is well known that the success of IVF/ICSI/IMSI treatment depends on many factors, including the age of the woman, the patient's medical history, as well as the quality of the gametes and embryos. Another important factor, which is often overlooked, might involve the embryo transfer (ET) technique itself. Cohen holds a "bad" ET responsible for a failed implantation in 30% of cases [1]. The Evidence Based Guidelines (EBG) recommends avoiding a difficult ET [2]. Various techniques and supporting devices are used, such as a soft catheter (e.g. Wallace catheter) for atraumatic transfers or external guidance with introduction of the catheter through an advanced sheath, while stylet, forceps, and tenaculum are used for difficult situations. It has been shown in the literature that complications, such as bleeding or tenderness, may play a role in pregnancy outcome and influence the interpretation of how difficult or easy an ET was [3, 4].

Another factor frequently discussed in relation to ETs are uterine contractions induced by transfer catheters or supporting devices used in the course of an ET. These contractions seem to particularly occur after difficult ETs and might interfere with embryo implantation [5, 6]. The impact of such contractions might even be more profound on day-3 as compared to day-5 ETs due to the influence of progesterone [7–9].

In our study, we retrospectively analyzed the outcome of 1,055 day-3 and day-5 ETs at our center in order to evaluate the impact of ET quality (quality 1–4, with ET quality 1 being the best) on the CPR and LBDR.

Material and methods

In the period between November 2005 and December 2009, 1,055 fresh ETs were evaluated following IVF/ICSI/IMSI at a single center. Informed consent was signed by all patients. The GnRH long protocol was predominantly applied with daily injections of triptorelin (Decapeptyl®, Ferring Arzneimittel, Vienna, Austria) 0.1 mg, beginning in the mid-luteal phase of the preceding cycle, for down-regulation of the pituitary gland. HMG (Merional®, IBSA, Lugano, Swiss; Menogon-(HP)®, Ferring Arzneimittel, Vienna, Austria) was used for follicle stimulation. Starting on day 6 or 7 of stimulation, a transvaginal scan for 2-D measurement of follicular size was performed every 2 to 3 days until the administration of 5,000–10 000 IU β -HCG (Pregnyl®; Organon, Wien, Austria or Brevactid®; Ferring Arzneimittel, Kiel, Germany) to induce final oocyte maturation was indicated. Transvaginal oocyte retrieval was conducted 35 h later. The obtained MII oocytes were fertilized and kept in culture (Global medium supplemented with 7.5% human serum albumin; LifeGlobal, Ontario, Canada) for 3 or 5 days before transfer. There were no changes in the culture conditions during the time period of the study. On the day of transfer the quality of the embryos was evaluated morphological and the best embryos were selected [10, 11]. Top embryos were classified on d3 as 6–8 cells A1 and B1 and on d5 as blastocyst 2–5 with grading for inner cell mass and trophectoderm AA, AB or BA. The FSH dose was titrated to the patient's response from day 6 onwards. Luteal phase support consisted of 3×2 mg estradiol valerate tablets (Progynova®; Bayer, Leverkusen, Germany) and 100 mg progesterone ampoules every 36 h i.m. (Prontogest®; IBSA, Lugano, Switzerland).

In a preceding cycle, the cervix of all women was dilated using a No. 5 Hegar dilator, and the length of the uterus cavity was mechanically measured by means of a uterine probe. Mucus was removed from the external uterine orifice prior to the ET with sterile cotton swabs. The catheter was flushed with IVF culture medium and the embryos were

aspirated with the help of a micrometer screw as standardized since two decades in our centers into the inner sheath of the catheter with 2–3 μ l culture medium and 2–3 μ l air before and behind the embryos and 2–3 μ l medium at the tip of the catheter. The physician with assistance of the embryologist rapidly transferred the embryos 0.5 to 1.0 cm beneath the fundus uteri. After ET the catheter was checked for retained embryos under the microscope. Difficult transfers were managed first with use of external guidance with introduction of the catheter through an advanced sheath, then with a stylet and as last option with the use of a tenaculum. Patients remained supine for 10 min after the ET.

All ETs, which were performed using a Wallace Embryo Replacement Catheter (Smiths Medical International Ltd, Hythe, Kent, UK), were always carried out by the same two physicians, who have many years of experience, together with the same two experienced biologists. There were no changes in the ET-technique during the time period of the study.

The quality of the ETs was categorized into one of 4 ET groups and assessed by a physician and a biologist together: ET 1=an atraumatic ET, entering into the uterus cavity only with the soft inner part of a catheter; ET 2=use of external guidance with introduction of the catheter through an advanced sheath; ET 3=probing of the cervix with a stylet; ET 4=necessity to grasp the portio vaginalis with a tenaculum prior to entering the uterine cavity.

Therapy outcome measures were blood or urinary β -HCG tests 17 days after oocyte retrieval, an ultrasound scan 7–10 days thereafter, as well as questionnaires on CPR and LBDR.

Differences in the CPR and LBDR were evaluated with Pearson's χ^2 -test. A two-tailed t-test was used to test for differences in the number of oocytes retrieved and fertilization rates, as well as differences resulting from the patients' age. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) software version 17.0 for Windows (SPSS Inc., USA).

Results

The ET rate was 95.8% (1,055 fresh ETs resulting from 1,113 oocyte retrievals). A total of 865 ETs were carried out on day 5 (81.1%) and 190 (17.8%) on day 3. Eleven transfers (0.93%) were performed on day 2, 4, or 6 and were not included in the study. The distribution of the four different types of transfers was equal among the various practitioners. As shown in Table 1, there was a slight but significant difference between day-3 and day-5 ETs in relation to the age of the patients. Significantly, more oocytes were retrieved in the day-5 transfer group (4.2 vs.

Table 1 Outcomes of embryo transfer on day 3 and day 5

	Day 3	Day 5	p-value
Number of ETs	190	865	
Age (mean±sd)	37.5±4.3	35.5±4.6	<0.001
Oocytes/ pickup (mean±sd)	4.2±2.8	10.2±4.8	<0.001
2PN (mean±sd)	1.8±0.9	7.0±3.6	<0.001
Embryos/transfer (mean±sd)	1.7±0.6	1.9±0.4	<0.001
Transfer with top-embryos	82 (43.2%)	358 (41.4%)	n.s.
ET 1	147 (77.4%)	644 (74.5%)	n.s.
ET 2	25 (13.2%)	125 (14.5%)	n.s.
ET 3	9 (4.7%)	65 (7.5%)	n.s.
ET 4	9 (4.7%)	31 (3.6%)	n.s.
β-HCG positiv	47 (24.5%)	402 (46.5%)	<0.001
Biochemical pregnancy	4 (8.5%)	31 (7.7%)	n.s.
Miscarriages	9 (19.1%)	74 (18.4%)	n.s.
Ectopic pregnancy	0	1 (0.1%)	n.s.
LBDR	34 (17.9%)	296 (34.2%)	<0.001

10.2), corresponding to a higher number of fertilized oocytes on day 1.

A mean number of 1.7 embryos compared to 1.9 were transferred on day 3 and day 5, respectively. No differences were found in the amount of top embryo transfers and the distribution of different ET techniques between the 4 groups. When analyzing pregnancy outcome, no differences were found in the occurrence of biochemical pregnancies and miscarriages (all definitions according to Zegers-Hochschild et al, 2009) either [12]. Notably, a statistically significant difference was found between day-3 and day-5 ETs concerning the LBDR (17.9% vs. 34.2%; p-value<0.001).

In the next step, we analyzed the impact of different ET techniques on pregnancy outcome. As shown in Table 2, no differences relating to the age of patients were seen, and, furthermore, no difference in the number and quality of embryos transferred was observed. When analyzing the CPR, we found that the highest percentage was obtained in those patients who had had an atraumatic ET (ET 1; 44.5%). A noteworthy decrease in the CPR (36%) was detected in patients with ET 2. Interestingly, a CPR of 40.5% was obtained in patients with ET 3. The lowest

percentage was found in the ET 4 group (32.5%). These results are also reflected in the LBDR. A significantly lower LBDR was observed in patients with ET 2 as compared to ET 1 (26.0% vs. 32.5%). The highest LBDR was found among patients with ET 3 (33.9%) and the lowest rate among patients with ET 4 (22.5%). No differences were found in the miscarriage rates between the groups.

Additionally, we assessed the impact of embryo quality on pregnancy outcome (data not shown). Patients who tested positive for β-HCG received a top embryo significantly more often (53.4%) than those with negative results (34.8%). There was also a significant difference in the age of patients with a positive pregnancy test (34.8 vs. 36.6; p-value<0.001).

Discussion

EBG recommendations include avoiding difficult ETs and the use of soft catheters [2]. Our data clearly show that the highest chance of pregnancy and live birth is with an atraumatic ET. One explanation for this might be that manipulations of the cervix (for example, with a tenaculum) might lead to uterine contractions and should be avoided [5]. Interestingly, the transfer of embryos with external guidance was accompanied by an 6.5% reduction in the CPR and a significant reduction in the LBDR as compared to ET 1. However, in this group a not significant reduction in the percentage of top-embryos was found which could contribute but not solely explain this observation. This result is surprising, since using a stylet (ET 3) resulted in a similar outcome as compared to an atraumatic ET. Although the group of patients receiving an ET 3 was rather small (approximately 6% of all patients) and might limit our findings, this result may still be of relevance. Possibly, external guidance carries a greater risk of transferring mucus and blood into the fundus in contrast to the smooth positioning of a stylet in the region of the cervix. However, results are still conflicting, with one study showing no negative effects either of mucus or blood inside or on the catheter or of retracted embryos on pregnancy outcome [13].

Table 2 Influence of different ET-techniques on CPR and LBDR

	ET 1	ET 2	ET 3	ET 4
Age (mean±sd)	36.0±4.6	35.7±4.7	35.7±4.7	35.8±4.6
Embryo/transfer (mean±sd)	1.9±0.5	1.8±0.5	1.9±0.4	1.9±0.4
Transfers with top-embryos	328/791 (41.5%)	54/150 (36.0%)	36/74 (48.6%)	22/40 (55.0%)
CPR	352/791 (44.5%)	54/150 (36.0%)	30/74 (40.5%)	13/40 (32.5%)
LBDR	257/791 (32.5%)	39/150* (26%)	25/74 (33.9%)	9/40 (22.5%)
Miscarriages	67/791 (8.5%)	12/150 (8.0%)	3/74 (4.1%)	1/40 (2.5%)

*p-value<0.05

In their analysis of 2,263 fresh day-3 ETs preceded by a dummy or mock transfer, Spandorfer et al. found ETs difficult in 4.68% of cases. These difficult ETs were reported to be negatively associated with the CPR [13]. Notably, Drakeley et al. were able to show in 2,295 ultrasound-guided ETs that grasping the portio vaginalis (1.7%) negatively affects the CPR (8% vs. 24%, 22%, and 20%, respectively, in the other three groups) [14]. These findings are in line with our results as regards the percentage of difficult ETs and the decrease in the CPR.

No differences in the biochemical or ongoing CPR and LBDR were reported in one study by Tur-Kaspa et al [15]. of 854 prospectively analyzed ETs, which were categorized into simple or difficult (need of cervix dilation or a repeated sequential ET). However, distinguishing solely between simple and difficult ETs might not be differentiated enough to detect differences in pregnancy outcome. In our study, we see the major decline in the CPR and LBDR between ET 1 and ET 2.

Generally, a significantly higher CPR was observed in the day-5 ET group. One explanation, which is also a limitation of this study, is that low responders were predominantly found in the day-3 collective. However, other reasons that contribute to these results might be that contractions possibly lead to an increase in implantation failures. Biervliet et al. believe that uterine contractions are less harmful for day-5 ETs compared to day-2 to day-3 ETs due to the uterus-relaxing effects of progesterone [7]. Fanchin et al. have shown earlier that uterus contractions significantly decrease 7 days after HCG application [8, 9]. It is noteworthy that differences in the CPR and LBDR between ET 1–4 were less pronounced in patients receiving a day-3 ET compared to those with a day-5 ET (data not shown). These findings strongly support the hypothesis that uterine contractions have a more pronounced effect on embryo implantation in day-5 ETs.

Furthermore, blastocyst culture of all embryos might contribute to our results. The selection of embryos on day 5 proved to be a much better technique for selecting the fittest embryos that are most likely to implant [16–18]. Angelini et al. showed that a higher experienced physician had significant higher CPR and implantation rate. In our study all ET were done by two physicians with many years of experience, together with the same two experienced biologists [19].

In conclusion, we found that the ET technique might have an important impact on the CPR and LBDR. Besides embryo quality, embryo selection (day 2–3 vs. day 5), and the age of patients, this may be a fourth important aspect to consider when planning treatment. If an atraumatic transfer is not possible, the primary use of a stylet instead of external guidance may have to be considered. However, further investigations will be required to confirm these findings. The mechanical measurement of uterine cavity

length in a preceding cycle or a mock transfer make it possible both to determine the ideal place for depositing the embryos and to avoid touching the fundus uteri. These techniques should be offered to all patients besides an ultrasound scan before starting therapy.

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Conflicts of interest None

References

1. Cohen J. Embryo replacement technology. San Francisco 31 Annual post graduate course. ASRM. 1989
2. Mains L, Van Voorhis BJ. Optimizing the technique of embryo transfer. *Fertil Steril*. 2010;94:785–90.
3. Kovacs GT. What factors are important for successful embryo transfer after in-vitro fertilization? *Hum Reprod*. 1999;14:590–2.
4. Lass A, Abusheikha N, Brinsden P. The effect of a difficult embryo transfer on the outcome of IVF. *Hum Reprod*. 1999;14:2417.
5. Lesny P, Killick SR, Robinson J, Raven G, Maguiness SD. Junctional zone contractions and embryo transfer: is it safe to use a tenaculum? *Hum Reprod*. 1999;14:2367–70.
6. Lesny P, Killick SR, Tetlow RL, Robinson J, Maguiness SD. Embryo transfer—can we learn anything new from the observation of junctional zone contractions? *Hum Reprod*. 1998;13:1540–6.
7. Biervliet FP, Lesny P, Maguiness SD, Killick SR. Ultrasound-guided embryo transfer maximizes the IVF results on day 3 and day 4 embryo transfer but has no impact on day 5. *Hum Reprod*. 2002;17:1131–2.
8. Fanchin R, Ayoubi J-M, Righini C, Olivennes F, Schönauer LM, Frydman R. Uterine contractility decreases at the time of blastocyst transfer. *Hum Reprod*. 2001;16:1115–9.
9. Fanchin R, Righini C, Olivennes F, Taylor S, De Ziegler D, Frydman R. Uterine contractility decreases at the time of blastocyst transfer. *Hum Reprod*. 1998;13:1968–74.
10. Gardner DK, Schoolcraft WB. In vitro culture of human blastocysts. In: Jansen R, Mortimer D, editors. Towards reproductive certainty: infertility and genetics beyond 1999. Chichester: Parthenon Press; 1999. p. 378–88.
11. Puissant F, Van Rysselberge M, Barlow P, Deweze J, Leroy F. Embryo scoring as a prognostic tool in IVF treatment. *Hum Reprod*. 1987;2:705–8.
12. Zegers-Hochschild F, Adamson GD, de Mouzon J, Ishihara O, Mansour R, Nygren K, Sullivan E, van der Poel S, on behalf of ICMART and WHO. The International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary on ART terminology, 2009†. *Hum Reprod*. 2009;11:2683–7.
13. Spandorfer SD, Goldstein J, Navarro J, Veeck L, Davis OK, Rosenwaks Z. Difficult embryo transfer has a negative impact on the outcome of in vitro fertilization. *Fertil Steril*. 2003;79:654–5.
14. Drakeley A, Jorgensen A, Sklavounos J, Aust T, Gazvani R, Williamson P, et al. A randomized controlled clinical trial of 2295 ultrasound-guided embryo transfers. *Hum Reprod*. 2008;23:1101–6.
15. Tur-Kaspa I, Yuval Y, Bider D, Levron J, Shulman A, Dor J. Difficult or repeated sequential embryo transfer do not adversely affect in-vitro fertilization pregnancy rates or outcome. *Hum Reprod*. 1998;13:2452–5.

16. Gardner DK, Schoolcraft WB, Wagley L, Schlenker T, Stevens J, Hesla J. A prospective randomized trial of blastocyst culture and transfer in in-vitro fertilization. *Hum Reprod.* 1998;12:3434–40.
17. Papanikolaou E, Camus M, Kolibianakis M, Van Landuyt L, Van Steirteghem A, Devroey P. In vitro fertilization with single blastocyst-stage versus single cleavage-stage embryos. *N Engl J Med.* 2006;354:1139–46.
18. Zech NH, Lejeune B, Puissant F, Vanderzwalmen S, Zech H, Vanderzwalmen P. Prospective evaluation of the optimal time for selecting a single embryo for transfer: day 3 versus day 5. *Fertil Steril.* 2007;88:244–6.
19. Angelini A, Brusco GF, Barnocchi N, El-Danasouri I, Pacchiarotti A, Selman HA. Impact of physician performing embryo transfer on pregnancy rates in an assisted reproductive program. *J Assist Reprod Genet.* 2006;23:329–32.