Reproductive BioMedicine Online (2010) **20**, 380–386

www.sciencedirect.com
www.rbmonline.com

ARTICLE

Factors affecting pregnancy in IVF: age and duration of embryo transfer

MT Cetin [a,*], Y Kumtepe [b], H Kiran [b], G Seydaoglu [c]

[a] *Cukurova University, Faculty of Medicine, Department of Gynecology and Obstetrics, Balcalı, Adana, Turkey;* [b] *Prof Dr M Turan Cetin IVF Center, Adana, Turkey;* [c] *Cukurova University, Faculty of Medicine, Department of Biostatistics, Balcalı, Adana, Turkey*

[*] Corresponding author. *E-mail address:* info@tupbebekmerkezi.net (MT Cetin).



After having worked extensively in the areas of infertility, endoscopic surgery (laparoscopy, hysteroscopy) and reproductive endocrinology in Turkey, Prof Dr M Turan Çetin has worked in Montpellier University, and St Joseph Hospital Montplaisir Clinique in Lyon in the fields of infertility, reproductive endocrinology, endoscopic surgery and IVF-embryo transfer. He is one of the first doctors to have performed endoscopic surgery in Turkey and his work resulted in the first IVF baby to be born in Adana, Turkey. He is a nationally renowned infertility specialist.

Abstract The aim of this retrospective study was to investigate factors affecting clinical pregnancy rates, especially age and duration of embryo transfer, in IVF/intracytoplasmic sperm injection cycles (1313 embryo transfers). Overall clinical pregnancy rate was 30.0% (n = 394). Clinical pregnancy rates were found to be 31.6% for the <44 s interval, 25.9% for the 45–59 s interval and 23.6% for the ≥60 s interval (P = 0.020). In the <35 year age group, clinical pregnancy rates were 35.1%, 29.9% and 30.6%, and in the ≥35 year age group, they were 26.9%, 21.0% and 13.4% (P = 0.013), respectively. According to logistic regression analyses, the odds of failed pregnancy increased by 1.61 times [95% confidence interval (CI) 1.07–2.41] for embryo transfer durations longer than 60 s and odds ratios of a failed pregnancy were 1.53 (95% CI 1.18–1.99) in the ≥35 year age group, 1.49 (95% CI 1.05–2.12) for fewer than five oocytes and 3.38 (95% CI 2.10–5.43) for fewer than two transferred embryos. In conclusion, to increase the likelihood of a successful pregnancy in women over 35 years of age, the duration of embryo transfer must be kept below 60 s. The number of oocytes obtained and the number of transferred embryos also play a role in the success of pregnancy.

© 2009, Reproductive Healthcare Ltd. Published by Elsevier Ltd. All rights reserved.

KEYWORDS: age, catheter, embryo transfer duration, prognostic factors

Introduction

Unsuccessful implantation after embryo transfer is still the most important problem of IVF. It has been estimated that implantation failure occurs in 85% of replaced embryos (Edwards, 1995). The most important factors influencing implantation rates are age, patient selection, type of protocol, uterine receptivity, gamete and embryo quality and transfer efficiency. Despite the fact that embryo transfer is the most important mechanical step of IVF, much less effort has been placed on improving it compared with other aspects of IVF (Salha et al., 2001; Schoolcraft, 2001; Wood et al., 2000). In recent years, however, interest has increased concerning the methodology of embryo transfer

1472-6483/$ - see front matter © 2009, Reproductive Healthcare Ltd. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.rbmo.2009.12.008

and catheter quality. A number of prognostic factors have been described for embryo transfer, such as atraumatic transfer, absence of blood or mucus in the transfer catheter, absence of bacterial contamination, using soft catheters, not touching the uterine fundus, uterocervical angle and using ultrasound guidance (Matorras et al., 2002; Sallam et al., 2002; Schoolcraft, 2001).

Implantation failure may be a result of uterine contractions (Fanchin et al., 1998) and endometrial damage (Marikiniti et al., 2003) caused by cervical manipulation during difficult transfers and touching of the catheter on the uterine fundus (Salha et al., 2001). It is also thought that embryos can be damaged when subject to environmental factors other than that of the human body and that the composition of catheters can have a detrimental effect on embryos (Kea et al., 2007; Matorras et al., 2004; Quinn, 2000). The aspect of duration of embryo transfer has been previously studied in respect to IVF success by Matorras et al. (2004) who have found that the longer the interval between loading and discharging of embryos, the lower the clinical pregnancy rate. The aim of the present study was to study the effect of factors affecting clinical pregnancy rates in IVF/intracytoplasmic sperm injection cycles, especially age and time employed in the performance of the transfer (the time interval between catheter loading and discharging of embryos).

Materials and methods

The data used in this study was collected from all the IVF/intracytoplasmic sperm injection cases performed at the study unit between January 2004 and January 2007. A total of 1313 IVF cycles (1306 patients) which underwent embryo transfer were included; however, frozen embryo and blastocyst transfer cycles were not included in the study. The two cycles of 7 patients who underwent IVF twice were included in the study as they did not become pregnant and a minimum of 6 months had passed between each treatment. Of the 1306 patients, 1184 had primary infertility while 122 had secondary infertility. None of the patients had had a previous IVF pregnancy.

The antagonist protocol was performed on 348 cycles while the long protocol was performed on 965 cycles. In the study unit, recombinant FSH (Puregon, Organon, Turkey, and Gonal F, Serono, Turkey), gonadotrophin-releasing hormone agonist (leuprolide acetate, Lucrin, Abbott Laboratories, Turkey) and gonadotrophin-releasing hormone antagonist (Cetrotide 0.25 mg, Serono, and Orgalutron 0.25 mg. Organon), were used for ovarian stimulation and recombinant human chorionic gonadotrophin (Ovitrelle, Serono) or urinary human chorionic gonadotrophin (Pregnyl 5000, Organon) were used for the triggering of ovulation. Oocyte retrieval was performed under ultrasound guidance 35 h after human chorionic gonadotrophin injection. The protocols of the embryology laboratory were performed under the Revised European Society for Human Reproduction and Embryology [ESHRE] Guidelines for Good Practice in IVF Laboratories (Magli et al., 2008). The scoring of high quality embryos was according to Veeck's published criteria (Veeck, 1999). Luteal phase support was achieved with 200 mg/8 h vaginal micronized natural progesterone (Progestan, Koçak Laboratories, Turkey).

A mock-transfer was performed on all patients before the start of the treatment (Mansour et al., 1990). Difficult mock-transfers were characterized by a difficult insertion of a Wallace catheter and/or the need for a tenaculum or anaesthesia. In difficult cases, an embryo difficult transfer (EDT) catheter was planned to be used in the embryo transfer. The need for general anaesthesia and use of a tenaculum was found to be required on only seven patients during the mock transfer and to evaluate the cavity of patients with difficult mock-transfers, a diagnostic hysteroscopy was performed before the start of the treatment. This way, the catheter and method to be used (map of cervical canal and whether anaesthesia was necessary) was determined before the day of embryo transfer. During embryo transfer, neither tenaculum nor anaesthesia were used in these patients as the use of an EDT catheter was sufficient. The average endometrial thickness on the day of the transfer was 12 mm. The day of embryo transfer for all patients was day 3 and only grade 1 and 2 embryos were transferred. The number of embryos transferred depended on the number of embryos obtained, a maximum of three embryos being allowed in Turkey irrespective of patient's age. The embryos of patients with more than three embryos were frozen. Quinn's Advantage Protein Plus Cleavage medium (Sage, USA) was used for all embryos.

A speculum was inserted into the vagina of the patient lying with a full bladder in the lithotomy position. The cervix was cleaned (G rinse, Vitrolife, USA) and cervical mucus was wiped off the external ostium. Mucus aspiration was rarely performed. After this, the endometrial lining was measured using ultrasound and the uterus and endometrium were shown on the screen to the patient. During this time, an EDT catheter 18 mm (Rocket UK) was used in patients who previously had a difficult mock-transfer and a Wallace Soft Catheter 18 mm (Smith, UK) was used in patients who previously had an easy mock-transfer. The outside sheath of the catheter was inserted up to the internal os being careful not to insert it into the cavity. The embryos were released under ultrasound guidance in the middle of the uterine cavity. A chronometer was started by one embryologist as soon as the embryos were loaded into the catheter and the chronometer was stopped as soon as the embryos were released from the catheter. The duration of embryo transfer (time interval the embryos remained in the catheter) was then recorded in the patient's file. In some patients with either easy or difficult mock-transfers, the insertion of the catheter into the cavity through the internal os was difficult during the embryo transfer causing longer embryo transfer durations. Dilatation was not performed on any patient. All embryo transfers were performed by the same clinican. Clinical pregnancy was defined by the visualization of a fetal heart beat at 7—8 weeks of amenorrhoea.

Variables such as age, infertility, protocol, length of stimulation, endometrial thickness, total oocyte number, total number of transferred embryos, difficulty of embryo transfer and duration of embryo transfer were collected. Embryo transfer duration was categorized according to age (above and below 35 years) and into three groups: (i) less than 44 s; (ii) 45—59 s; and (iii) 60 s or more, which was subdivided into two groups, less than 59 s and 60 s or more.

Statistical analysis

Normality was checked for each continuous variable. Normally distributed values were expressed as mean ± SD, number (%) and others as median (range), where appropriate. A *P*-value <0.05 was considered as significant. The categorical variables between the groups were analysed by using the chi-squared test. The odds ratio (OR) and its 95% confidence interval (95% CI) were estimated using the Statistical Package for Social Sciences version 12.0 (SPSS, USA). Logistic regression analysis was used to calculate the OR of failed pregnancy in subjects after adjustment of risk categories such as age, number of oocytes, duration of embryo transfer, number of transferred embryos and difficulty of transfer as independent variables. Independent variables were selected as the variables which had *P*-values which were <0.2 according to the clinically important factors analysis for each variable. In addition, a clinically important factor such as difficulty of transfer was also selected as an independent variable. Results of difficulty of transfer were analysed by chi-squared trend analysis. Tables showing the subgroup analysis were not included in the article because of low numbers.

Results

The data were divided into two groups: pregnant (success) and not pregnant (failure). As seen in **Table 1**, these two groups were then compared according to age, type of infertility, type of protocol, length of stimulation, endometrial thickness (cut off >7 mm), total oocyte number, total number of transferred embryos, difficulty of embryo transfer, embryo transfer duration and embryo transfer duration interval. Age (*P* = 0.0001), number of transferred embryos (*P* = 0.001)

Table 1 Factors related to achieving pregnancy.

Factor	*Non-pregnant (failure)* (n = *919*)	*Pregnant (success)* (n = *394*)	P-*value*
Age			
<35 years	497 (69.8)	256 (34.0)	
≥35 years	422 (75.4)	138 (24.6)	0.0001
Infertility			
Primary	826 (69.8)	358 (30.2)	
Secondary	89 (73.0)	33 (27.0)	
Not known	4 (57.1)	3 (42.9)	NS
Protocol			
Antagonist	244 (70.1)	104 (29.9)	
Long	675 (69.9)	290 (30.1)	NS
Length of stimulation (days)	9.9 ± 2.6; 10 (1–26)	9.8 ± 2.4; 10 (1–26)	NS
Endometrial thickness	12.2 ± 1.9; 12 (7–23)	12.3 ± 1.8; 12 (4–23.5)	NS
Total number of oocytes	12.0 ± 8.0; 10 (1–42)	12.6 ± 7.9; 12 (1–44)	NS
Number of oocytes			
Fewer than five (poor responder)	228 (73.5)	82 (26.5)	NS
Five or more (normal)	691 (68.9)	312 (31.1)	
Number of transferred embryos			
One embryo	154 (85.1)	27 (14.9)	
Two embryos	195 (74.4)	67 (25.6)	
Three embryos	570 (65.5)	300 (34.5)	0.001
Ease of transfer			
Easy	886 (70.2)	376 (29.8)	
Difficult	33 (64.7)	18 (35.3)	NS
Duration of embryo transfer (s)	37.7 ± 25.7; 30 (10–171)	35.5 ± 22.1; 30 (10–140)	NS
Embryo transfer duration interval (s)			
≤44	690 (68.4)	319 (31.6)	
45–59	103 (74.1)	36 (25.9)	
≥60	126 (76.4)	39 (23.6)	0.020
Total	919 (70.0)	394 (30.0)	

Values are expressed as number (%) or mean ± SD; median (range). NS = not statistically significant.

Table 2 Relationship between the transfer duration intervals and difficulty of transfer according to the age subgroup and analyses of achieving pregnancy.

	Age <35 years			*Age ≥35 years*			*Total*		
	Failed pregnancy	*Pregnancy*	*OR*	*Failed pregnancy*	*Pregnancy*	*OR*	*Failed pregnancy*	*Pregnancy*	*OR*
Duration of embryo transfer (s)									
<44	375 (64.9)	203 (35.1)	1 (reference)	315 (73.1)	116 (26.9)	1 (reference)	690 (68.4)	319 (31.6)	1 (reference)
45–59	54 (70.1)	23 (29.9)	1.3 (0.7–2.2)	49 (79.0)	13 (21.0)	1.4 (0.7–2.8)	103 (74.1)	36 (25.9)	1.3 (0.9–2.0)
>60	68 (69.4)	30 (30.6)	1.2 (0.8–2.0) NS[a]	58 (86.6)	9 (13.4)	2.4 (1.1–5.3) 0.013[b]	126 (76.4)	39 (23.6)	1.5 (1.0–2.3) 0.020[b]
Duration of embryo transfer (s)									
<60	429 (65.5)	226 (34.5)	1.2 (0.7–1.9)	364 (73.8)	129 (26.2)	2.3 (1.1–4.7)	793 (69.1)	355 (30.9)	1.4 (0.9–2.1)
>60	68 (69.4)	30 (30.6)	NS[a]	58 (86.6)	9 (13.4)	0.023[a]	126 (76.4)	39 (23.6)	NS[a]
Difficulty of transfer									
Difficult	18 (54.5)	15 (45.5)	0.6 (0.3–1.2)	15 (83.3)	3 (16.7)	1.7 (0.5–5.8)	33 (64.7)	18 (35.3)	0.8 (0.4–1.4)
Easy	479 (66.5)	241 (33.5)	NS[a]	407 (75.1)	135 (24.9)	NS[a]	886 (70.2)	376 (29.8)	NS[a]
Total	497 (66.0)	256 (34.0)		422 (75.4)	138 (24.6)		919 (70.0)	394 (30.0)	

Values are number (%) or OR (95% confidence interval); NS = not statistically significant; OR = odds ratio.
[a] *P*-value for chi-squared test.
[b] *P*-value for chi-squared trend analyses.

and embryo transfer duration intervals ($P = 0.02$) led to statistically significant differences in clinical pregnancy rates.

Total clinical pregnancy rates were found to be 31.6% for the <44 s interval, 25.9% for the 45–59 s interval and 23.6% for the ≥60 s interval ($P = 0.020$). The relationship between embryo transfer duration intervals and clinical pregnancy rate was analysed according to age groups (**Table 2**). Clinical pregnancy rates were found to be 35.1%, 29.9% and 30.6% in the <35 year age group, and 26.9%, 21.0% and 13.4% in the ≥35 year age group ($P = 0.013$), respectively. In addition to that, the embryo transfer duration was classified into groups of <60 s and ≥60 s and odds ratios were calculated for these according to age groups. The total clinical pregnancy rate was lower in embryo transfer durations ≥60 s than in durations <60 s (OR 1.4, 95% CI 0.9–2.1); however, this was not found to be statistically significant. The clinical pregnancy rate was lower in the ≥35 age group with duration ≥60 s and the difference was found to be statistically significant (OR 2.3, 95% CI 1.1–4.7; $P = 0.023$). According to age subgroups, no relationship was found between difficulty of transfer and clinical pregnancy rate.

When endometrial thickness was evaluated as categorical data such as using 7 mm as a cut-off value, the numbers become too low for statistical analyses. Therefore, this parameter was used as a continuous variable.

The results of the logistic regression analysis are presented in **Table 3**. Age, number of oocytes, duration of embryo transfer and number of transferred embryos were significantly associated with failed pregnancy. The odds of failed pregnancy increased by 1.61 times (95% CI 1.07–2.41) for embryo transfer durations longer than 60 s. The odds ratios were found to be 1.53 (95% CI 1.18–1.99) for the age group above 35, 1.49 (95% CI 1.05–2.12) for the number of oocytes less than five (poor responder) and 3.38 (95% CI 2.10–5.43) for the number of transferred embryos less than two.

Number of oocytes or embryos were related with age. But these factors were already evaluated and the result was already standardized with these factors by the multivariate analyses.

Discussion

Many studies have shown the importance of avoiding factors which negatively affect embryo implantation. Factors associated with failed implantation include the clinician and embryologist's experience, catheter choice, endometrial damage, uterine contractions, presence of blood or bacteria in the catheter and embryo misplacement (Coroleu et al., 2002; Fanchin et al., 1998; Lesny et al., 1998; Marikiniti et al., 2003). Difficult or sequential embryo transfers do not adversely affect IVF pregnancy rates or outcome (Tur Kapsa et al., 1998); however, blood found outside, but not inside, the transfer catheter after embryo transfer is associated with lower rates of embryo implantation and clinical pregnancy. An emphasis on atraumatic transfer techniques with prevention of bleeding at the time of embryo transfer should contribute to improved pregnancy outcome (Goudas et al., 1998). In another study, the difficult transfers were associated with lower clinical pregnancy rates compared with easy transfers, although the difference was not statistically significant (Sallam et al., 2002). In the present study, the difference in clinical pregnancy rates between difficult and easy embryo transfers was not statistically significant.

Ultrasound-guided embryo transfer was performed on all cases. Ultrasound-guided embryo transfer was first suggested in 1985 (Sallam et al., 2002). Ultrasound was also used to confirm the correct deposition of embryos into the uterine fundus. The results showed that this new technique improves both clinical pregnancy and implantation rates (Sallam et al., 2002). Although Drakely et al. showed no difference between ultrasound guidance and clinical touch in their paper (Drakeley et al., 2008), the use of ultrasound helps in the insertion of the catheter by showing the uterocervical angle and the internal os, avoiding touching the fundus and damaging the endometrium during the insertion of the guided catheter (EDT) and seeing where the embryos are released. However, despite the use of ultrasound, sometimes the insertion of the catheter into the cavity through the internal os can be difficult. Some transfer durations were longer than anticipated because of difficulties encountered during the embryo transfer, such as the bladder of the

Table 3 Multivariate analysis of factors affecting pregnancy.

Independent variables	*OR*	*95.0% CI*	*P-value*
Age ≥35 years	1.53	1.18–1.99	0.001
Fewer than five oocytes (poor responder)	1.49	1.05–2.12	0.025
Difficult transfer	0.66	0.35–1.23	NS
Duration of embryo transfer (s)			
≤44	Reference		0.038
45–59	1.32	0.88–1.99	NS
≥60	1.61	1.07–2.41	0.021
Number of transferred embryos			
Three embryos	Reference		0.0001
Two embryos	1.71	1.21–2.40	0.002
One embryo	3.38	2.10–5.43	0.0001

CI = confidence interval; NS = not statistically significant; OR = odds ratio.

patient being too full, the patient being too nervous and/or the clinician not being able to give the catheter the right utero-cervical angle. The embryos in these embryo transfers therefore remained in the catheter for a longer time period. This was especially seen in patients with an easy previous mock-transfer. In cases with difficult mock-transfers, the embryo transfer procedure was started as soon as the embryos were inserted into the catheter to limit the time the catheter remained in the uterine cavity. However, despite this, embryo transfer durations were sometimes higher in this group also due to the above causes.

Many studies have also shown the detrimental effect of environmental factors such as light, heat, polyvinyl chloride and polychlorinated biphenyls on oocytes and embryos (Cohen et al., 1997; Evans et al., 1999; Hunter et al., 1988; Pocar et al., 2001; Rocha et al., 1998; Smith, 1993). The importance of keeping embryos close to 37°C and between specific oxygen and carbon dioxide concentration ranges is well known (Bavister, 1995; Cohen et al., 1997). Many technological devices have been designed to protect oocytes and embryos from environmental damage during manipulation and storage. However, it is considerably difficult to control environmental factors during loading and discharging of embryos into and from the catheter. Therefore, it is speculated that pregnancy rates decrease with longer time intervals spent in the catheter. This has been shown in Matorras et al. (2004)'s study. In the present study, transfer durations had an effect on the clinical pregnancy rates of women below 35 years of age; however, this was not statistically significant (34.5% and 30.6% for time intervals $<$60 s and $\geq$60 s respectively). However, clinical pregnancy rates of women above 35 years of age were significantly affected. The odds of no pregnancy achieved increased by 1.61 times (95% CI 1.07–2.41) for embryo transfer durations longer than 60 s. The odds ratio was found to be 1.53 (95% CI 1.18–1.99) for the age group above 35. This supports the theory that gametes and embryos of women above the age of 35 are more sensitive to environmental factors than gametes and embryos of younger women. In addition to that, the odds ratio was 1.49 (95% CI 1.05–2.12) for fewer than five oocytes (poor responder), and 3.38 (95% CI 2.10–5.43) for less than two transferred embryos. The possibility of finding fewer than five oocytes in patients aged above 35 was more than the patients aged below 35. Because of low numbers of oocytes, the embryo numbers were also lower. According to multivariate analysis, both oocyte and transferred embryo numbers can be thought as factors increasing pregnancy failure in women above 35 years of age.

In conclusion, embryo transfer is one of the most important steps of IVF and this has been shown in many recent studies. It is known that the depth of placement of embryos in the uterine cavity, their placement in the middle of the cavity, embryo quality, the embryo transfer technique, the use of ultrasound, the type of catheter used and the presence of blood on the catheter are all important factors in the success of pregnancy and implantation. In addition to that, because embryos are negatively affected by external factors, especially in patients above the age of 35, keeping the time that the embryos spend between the incubator and the uterine cavity to a minimum will be important in the success of IVF.

Factors that might cause a longer embryo transfer are the patient's bladder being too full, the patient being too nervous because of inadequate psychological support (partner's presence, acupuncture, etc.), the clinican's inexperience, the performance of the transfer at a busy time of day and not performing the mock-transfer before the start of the treatment. The embryo transfer duration can be lowered if the clinician and embryologist have experience using the catheter, if the mock transfer is performed before the start of the treatment and if the distance between the incubator and uterus is short (laboratory to transfer room). The performance of all transfers on calm patients with the use of EDT catheter and under ultrasound guidance without passing the internal os will lower the embryo transfer duration and therefore the embryos, especially embryos of women above the age of 35, will be less affected by environmental factors.

Acknowledgements

The authors would like to thank Kristin Micaleff Botros for her help with the writing of the article.

References

Bavister, B.D., 1995. Culture of preimplantation embryos: facts and artifacts. Hum. Reprod. Update 1, 91–148.

Cohen, J., Gilligan, A., Esposito, W., Schimmel, T., Dale, B., 1997. Ambient air and its potential effects on conception in vitro. Hum. Reprod. 12, 1742–1749.

Coroleu, B., Barri, P.N., Carreras, O., Martínez, F., Parriego, M., Hereter, L., Parera, N., Balasch, J., 2002. The influence of the depth of embryo replacement into the uterine cavity on implantation rates after IVF: a controlled, ultrasound-guided study. Hum. Reprod. 17, 341–346.

Drakeley, A.J., Jorgensen, A., Sklavounos, J., Aust, T., Gazvani, R., Williamson, P., Kingsland, C.R., 2008. A randomized controlled clinical trial of 2295 ultrasound-guided embryo transfers. Hum. Reprod. 23, 1101–1106.

Edwards, R.G., 1995. Clinical approaches to increasing uterine reseptivity during human implantation. Hum. Reprod. 10 (Suppl. 2), 60–66.

Evans, J., Wells, C., Hood, K., 1999. A possible effect of different light sources on pregnancy rates following gamete intra-Fallopian transfer. Hum. Reprod. 14, 80–82.

Fanchin, R., Righini, C., Olivennes, F., Taylor, S., de Ziegler, D., Frydman, R., 1998. Uterine contractions at time of embryo transfer alter pregnancy rates after in vitro fertilization. Hum. Reprod. 13, 1968–1974.

Goudas, V.T., Hammitt, D.G., Damario, M.A., Session, D.R., Singh, A.P., Dumesic, D.A., 1998. Blood on the embryo transfer catheter is associated with decreased rates of embryo implantation and clinical pregnancy with the use of in vitro fertilization – embryo transfer. Fertil. Steril. 70, 878–882.

Hunter, S.K., Scott, J.R., Hull, D., Urry, R.L., 1988. The gamete and embryo compatibility of various synthetic polymers. Hum. Reprod. 50, 110–116.

Kea, B., Gebhardt, J., Watt, J., Westphal, L.M., Lathi, R.B., Milki, A.A., Behr, B., 2007. Effect of reduced oxygen concentrations on the outcome of in vitro fertilization. Fertil. Steril. 87 (1), 213–216.

Lesny, P., Killick, S.R., Tetlow, R.L., Robinson, J., Maguiness, S.D., 1998. Embryo transfer – can we learn anything from the observation of junctional zone contractions? Hum. Reprod. 13, 1540–1546.

Magli, C., Van Den Abbeel, E., Lundin, K., Royere, D., Van der Elst, J., Gianoroli, L., 2008. Revised ESHRE guidelines for good practice in IVF laboratories. Hum. Reprod. 23 (6), 1253–1262.

Mansour, R., Aboulghar, M., Serour, G., 1990. Dummy transfer: a technique that minimizes the problems of embryo transfer and improves the pregnancy rate in human in vitro fertilization. Fertil. Steril. 54, 678–681.

Marikiniti, K., Mathews, T., Shuter, S., Brindsen, P., 2003. Hysteroscopic findings after mock embryo transfer. Abstracts Book for the 19th ESHRE Annual Meeting, Madrid, June–July 2003. Hum. Reprod. 18 (Suppl. 1), 39–44.

Matorras, R., Mendoza, R., Exposito, A., Rodriguez-Escudero, F.J., 2004. Influence of the time interval between embryo catheter loading and discharging on the success of IVF. Hum. Reprod. 19 (9), 2027–2030.

Matorras, R., Urquijo, E., Mendoza, R., Corcóstegui, B., Expósito, A., Rodríguez-Escudero, F.J., 2002. Ultrasound-guided embryo transfer improves pregnancy rates and increases the frequency of easy transfers. Hum. Reprod. 17, 1762–1766.

Pocar, P., Perazzoli, F., Luciano, A.M., Gandolfi, F., 2001. In vitro reproductive toxicity of polychlorinated biphenils: effects on oocyte maturation and development competence in the cattle. Mol. Reprod. Dev. 58, 411–416.

Quinn, P., 2000. Review of media used in ART laboratories. J. Androl. 21 (5), 610–615.

Rocha, A., Randel, R.D., Broussard, J.R., Lim, J.M., Blair, R.M., Roussel, J.D., Godke, R.A., Hansel, W., 1998. High environmental temperature and humidity decrease oocyte quality in Bos taurus but not in Bos indicus cows. Theriogenology 49, 657–665.

Salha, O.H., Lamb, V.K., Balen, A.H., 2001. A postal survey of embryo transfer practice in the UK. Hum. Reprod. 16, 686–690.

Sallam, H.N., Agameya, A.F., Rahman, A.F., Ezzeldin, E.F., Sallam, A.N., 2002. Ultrasound measurement of the uterocervical angle before embryo transfer: a prospective controlled study. Hum. Reprod. 17 (7), 1767–1772.

Schoolcraft, W.B., 2001. Embryo transfer. In: Gardner, D.K., Weissman, A., Howles, C.M., Shoham, Z. (Eds.), Textbook of Assisted Reproductive Techniques: Laboratory and Clinical Perspectives. Martin Dunitz, London, UK, pp. 623–626.

Smith, L.C., 1993. Membrane and intracellular effects of ultraviolet irradiation with Hoechst 33342 on bovine secondary oocytes matured in vitro. J. Reprod. Fertil. 99, 39–44.

Tur Kapsa, I., Yuval, Y., Bider, D., Levron, J., Shulman, A., Dor, J., 1998. Difficult or repeated sequential embryo transfers do not adversely affect in-vitro fertilization pregnancy rates or outcome. Hum. Reprod. 13 (9), 2452–2455.

Veeck, L.L., 1999. An Atlas of Human Gamete and Conceptuses: An Illustrated Reference for Assisted Reproductive Technology. Panthenon Publishing, NY, USA.

Wood, G.E., Batzer, F.R., Go, J.K., Gutmann, J.N., Corson, S.L., 2000. Ultrasound-guided soft catheter embryo transfers will improve pregnancy rates in in-vitro fertilization. Hum. Reprod. 15, 107–112.

Declaration: The authors report no financial or commercial conflicts of interest.

Received 9 March 2009; refereed 18 June 2009; accepted 23 November 2009.