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Article

Antibubble trajectory during embryo transfers in donor egg IVF does not predict success

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Abstract

Transient motion of embryo transfer-associated antibubbles was observed. This prospective study was performed to determine if this antibubble movement can predict a successful outcome. Transabdominal ultrasound-guided embryo transfers were performed in 187 recipients receiving identical hormone replacement therapy. All embryo transfers were performed by the first author, using the Sureview embryo transfer catheter, in 30 µl of culture media. Observation was made of the catheter placement in relation to the endometrial surface and uterine fundus during embryo transfer. Ultrasound-guided tracking of antibubble within the uterine cavity was done immediately after the piston was depressed at the time of embryo deposition. The antibubble movement was upwards (group A) in 104 embryo transfers and downwards (group B) in 83 transfers. No movement of embryo-associated air out of the uterine cavity, either into the cervix or the intramural portion of the Fallopian tube, was observed. The clinical pregnancy rate was similar in both groups: 47.12% in group A versus 45.78% in group B. The total implantation rate/embryo transferred was 19.34% in group A compared with 20.07% in group B. The movement of the embryo transfer-associated antibubble is unlikely to be a factor in predicting success in donor egg IVF cycles.

Keywords: *antibubble, donor egg IVF, embryo-associated air, embryo transfer, recipients, transabdominal ultrasound guidance*

Introduction

Embryo transfer catheters and the transfer technique itself have been continuously evolving. Initially, intrauterine embryo transfers were performed blindly, but transabdominal ultrasound (Strickler *et al.*, 1985; Leong *et al.*, 1986) and, more recently, vaginal ultrasound guidance (Anderson *et al.*, 2002) have added more consistency to the procedure. Ultrasound-guided embryo transfer during an IVF cycle was initially reported during the mid-1980s (Strickler *et al.*, 1985; Leong *et al.*, 1986) and has gradually become an integral part of the embryo transfer technique for many IVF clinics. Furthermore, several transfer catheters with stronger ultrasound reflection have been developed (Coroleu *et al.*, 2006). The combination of a modern catheter and ultrasound allows visual monitoring of the entire embryo transfer process, while navigating embryo placement within the uterine cavity with high precision. This allows an examination of the relationship between the site of embryo placement and the outcome (Matorras *et al.*, 2002; Cavagna *et al.*, 2006). It was demonstrated, for example, that optimal results require embryo placement within 2 cm from the uterine fundus (Oliveira *et al.*, 2004).

Ultrasonographic guidance has many potential advantages. It facilitates placement of soft catheters, avoids touching the fundus, confirms that the catheter is beyond the internal os in cases of an elongated cervical canal, and allows direction of the catheter along the contour of the endometrial cavity, thereby avoiding disruption of the endometrium, plugging of the catheter tip with endometrium and instigation of bleeding. Ancillary advantages include assessment of the ovaries and presence of excessive peritoneal fluid volume to confirm that the risk for the ovarian hyperstimulation syndrome is not so great as to preclude embryo transfer. Fluid in the endometrial cavity can also be ruled out. Furthermore, the full bladder required to perform transabdominal ultrasonographic guidance is itself helpful in straightening the cervical uterine access and improving pregnancy rates (Sundstrom *et al.*, 1984; Lewin *et al.*, 1997). In cases of pregnancy achieved through embryo transfer, approximately 80% of embryos implant in areas to which they initially are transferred and the exact area of embryo placement in the uterine cavity can be localized precisely by transabdominal ultrasound-guided embryo transfers (Baba *et al.*, 2000).

Recent literature suggests that a shallow embryo transfer improves pregnancy rates (Pope *et al.*, 2004; Pacchiarotti *et al.*, 2007). But the embryos do not seem to retain their place of deposition at the time of embryo transfer. A transient motion of the embryo transfer-associated antibubbles (droplets of the culture media surrounded by a thin film of air introduced during the embryo transfer catheter loading process) was observed in a prospective study to determine if this antibubble movement at the time of embryo transfer can predict a successful outcome.

Materials and methods

Transabdominal ultrasound-guided embryo transfers were performed in 187 consecutive donor egg recipients receiving identical hormone replacement therapy. The use of donor oocyte recipients acts to exclude variables relating to embryo quality based on age and causation of infertility. All embryo transfers were done by the same physician using the same embryo transfer catheter (SureView, Wallace, UK), the same culture media volume and the same catheter loading technique. Observation was made of the embryo transfer catheter placement in relation to the endometrial surface and uterine fundus during embryo transfer. Ultrasound-guided tracking of embryo-associated air within the uterine cavity was done immediately after the piston was depressed at the time of embryo deposition. The immediate first 5 s after the piston was depressed were observed.

Results

The antibubble either moved up (Group A) or down (Group B) immediately after the piston was depressed at the time of embryo deposition (**Figures 1** and **2**). The endometrial thickness, the number of embryos transferred and the number of Grade A embryos was not significantly different in the two arms (**Table 1**). The air-bubble movement was upwards (Group A) towards the fundus in 104 embryo transfers compared with a downward movement (Group B) in 83 transfers. No movement of embryo-associated air out of the uterine cavity, either into the cervix or the intramural portion of the Fallopian tube, was seen. There were no difficult transfers in either group. The clinical pregnancy rate was similar in both groups: 47.12% in Group A versus 45.78% in Group B. The multiple gestation rate/embryo transfer was 18.27% in Group A versus 19.28% in Group B. The total implantation rate/embryo transferred was 19.34% in Group A compared with 20.07% in Group B (**Table 2**).

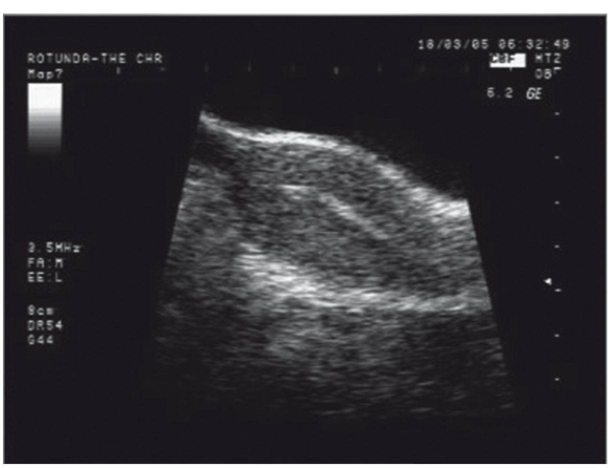

Figure 1. Antibubble is clearly seen near the fundal region of the uterine cavity. Part of the Sureview catheter is also seen in this ultrasonographic plate.

Figure 2. Antibubble seen in the uterine midcavity immediately following embryo transfer.

Table 1. Patient and embryo characteristics of the air-bubble movement groups.

	Group A – movement up (n = *104)*		*Group B – movement down* (n = *83)*	
	Average	*SD*	*Average*	*SD*
Age (years)	36.08	6.05	36.00	5.67
Embryos transferred (*n*)	3.81	0.51	3.83	0.76
Embryo grade				
A (*n*)	3.22	1.17	3.27	1.17
B (*n*)	0.46	0.88	0.59	0.96
C (*n*)	0.02	0.16	0.06	0.40
Endometrial thickness (mm)	9.18	1.42	9.66	1.49

SD = standard deviation.
There were no statistically significant differences between the two groups.

Table 2. Clinical outcomes in the air-bubble movement groups.

	Group A – movement up	*Group B – movement down*
Cycles (*n*)	104	83
Clinical pregnancy rate (%)	47.1	45.8
Biochemical pregnancy rate (%)	4.8	2.4
Ectopic pregnancy rate (%)	1.0	0
Total implantation rate/embryo transferred (%)	19.3	20.1
Abortion rate (%)	1.0	3.6
Multiple gestation rate (%)	18.3	19.3

There were no statistically significant differences between the two groups.

Discussion

To date, the use of air brackets is controversial and the evidence to support its use or avoidance is lacking. Some clinicians prefer the use of air brackets so that the embryo-containing media is easily identified in the catheter and on ultrasound (Confino *et al*., 2007). In addition, it has a psychological benefit for both the clinician and patient. The physician is guaranteed that the embryos will not be released from the catheter prematurely before proper placement in the uterus (Marek *et al*., 2004) or move up towards the syringe, therefore increasing the risk of being retained. Also the added visibility on ultrasound helps to detect the embryo catheter tip, in order to allow proper distancing from the uterine fundus. As for the patients, they are given the added comfort of visualizing the embryo-containing droplet on ultrasound through the detection of the two air bubbles surrounding the media.

Nassar *et al*. (2002) conducted a study to evaluate the impact of ultrasonographic-based embryo shift following uterine embryo transfer on reproductive outcome. Uterine embryo transfer was performed under transabdominal ultrasound guidance. The distance between the cephalic tip of the echogenic drop and the top of the endometrial cavity was measured immediately following embryo transfer (d1) and repeated 10–15 min later (d2). Embryo shift (d) represents the change in direction and distance between the two measurements. Based on embryo shift, patients were divided into three groups: Group 1 ($n = 17$) with d = 0, Group 2 ($n = 31$) with d = 0.1–3 mm and Group 3 ($n = 31$) with d = 3 mm. There was no statistically significant difference in age, stimulation and down-regulation protocol, number of eggs retrieved, number of embryos transferred, type of catheter used, ease of transfer and loading method between the three groups. Miscarriage and clinical, ongoing and multiple pregnancy rates were not statistically different between the three groups. Implantation and multiple pregnancy rates were 11.3% and 22.2% in Group 1, respectively. The difference was significant when compared with Group 2 (27.1%, 37.5%) and Group 3 (28% and 66.7%). The authors concluded that the direction of shift in the ultrasonographic echogenic drop following uterine transfer does seem to affect implantation rate. Careful and slow withdrawal of the transfer catheter might avoid downward shift of the transferred embryos and improve implantation rate.

Woolcott and Stanger (1998) investigated whether standing upright shortly after embryo transfer has any potential to affect the position of embryos transferred to the uterine cavity during IVF treatment. This was assessed by ultrasound-guided tracking of the embryo-associated air within the uterine cavity and a prospective study of 93 patients undergoing 101 consecutive embryo transfers in an IVF programme was carried out. Transvaginal ultrasound-guided embryo transfer was performed with a second ultrasound in standing position immediately after transfer, allowing the movement of embryo-associated air to be assessed. There was no movement in 94.1% (95/101) of transfers, movement of <1 cm in 4.0% (4/101) of transfers and movement of 1–5 cm in 2.0% (2/101) of transfers. No movement of embryo-associated air out of the uterine cavity, either into the cervix or the intramural portion of the Fallopian tube, was seen. The authors summarized that standing shortly after embryo transfer does not play a significant role in the final position of embryo-associated air and is unlikely to be a factor in determining the position of embryos transferred to the uterine cavity during treatment with IVF.

Knutzen *et al.* (1992) demonstrated the potential risk of contrast medium being expelled from the uterine cavity along the path of a transfer catheter. It was unfortunate, however, that the volume of contrast medium injected in this study was substantially more than is usually used. Krampl *et al.* (1995) have demonstrated that the use of embryo transfer-associated air bubbles does not affect pregnancy rates adversely in IVF therapy and has some potential advantages of minimizing capillary action within the narrow diameter catheters used for embryo transfer. All of these studies have the limitation of not actually tracking the movement and position of embryo-associated phenomena. They are, however, valuable in adding to current understanding. Transvaginal ultrasound tracking of a transfer-associated air bubble also clearly has limitations, as the embryos themselves are not observed. It would however appear to be an in-vivo direct observational method capable of providing insight into potential embryo movement on standing after transfer. It is impossible, however, to assess whether the embryos moved independently of the intrauterine air bubble. The laboratory experience in this study has been that on occasions embryos will attach to air bubbles and be moved with them upon manipulation. Embryos may alternatively move in the opposite direction. For example, they may settle to the lower endometrial surface while the air bubble rises. Therefore the direction of movement of the air bubble may not necessarily reflect the direction of movement of embryos. These results are consistent with the hypothesis that gravity is unlikely to be a significant force affecting the position of embryos within the uterine cavity following transfer.

Air bubble location following embryo transfer is presumed to be the placement spot of embryos. The purpose of a recent study was to document endometrial air bubble position and migration following embryo transfer (Confino *et al.*, 2007). A total of 88 embryo transfers were performed under abdominal ultrasound guidance in two countries by two authors. A single or double air bubble was loaded with the embryos using a soft, coaxial, end-opened catheters. The embryos were slowly injected 10–20 mm from the fundus. Air bubble position was recorded immediately, 30 min later and when the patient stood up. Bubble marker location analysis revealed a random distribution without visible gravity effect when the patients stood up. The bubble markers demonstrated splitting, moving in all directions and dispersion. Air bubbles move and split frequently after embryo transfer with the patient in the horizontal position, suggestive of active uterine contractions. Bubble migration analysis supports a rather random movement of the bubbles and possibly the embryos. Standing up changed somewhat the bubble configuration and distribution in the uterine cavity. Gravity-related bubble motion was uncommon, suggesting that horizontal rest after embryo transfer may not be necessary. Confino *et al.* (2007) concluded that the very random bubble movement observed in this two-centre study suggests that a large 'window' of embryo placement maybe present. This report challenges the common belief that a very accurate ultrasound-guided embryo placement is mandatory and is complementary to the conclusions previously made by the authors.

Conclusion

The movement of the embryo transfer-associated antibubble or the final position of embryo-associated air is unlikely to be a factor in predicting success in donor egg IVF recipient treatment cycles. Both the patient and her partner have the opportunity to be involved and directly visualize the transfer of their embryos to the uterine cavity with ultrasonographic guidance. It allows their involvement and commentary on the process and the psychological security of the satisfactory completion of the technical components of their treatment cycle.

Embryo transfer is a mechanical procedure, in which two materials (e.g. liquid and air) are transferred in sequence into the uterine cavity, which is filled with a more viscous fluid. A recent study showed that the composition of the transferred volume determines the spreading pattern of the transferred matter within the uterine cavity and, accordingly, controls the transport of the embryos (Eytan *et al.*, 2007). The objective of a recent systematic review was to determine the beneficial or detrimental effect of using air bubbles to bracket the embryo-containing medium during embryo transfer. The author concluded that there is insufficient evidence to suggest that the fluid-only method is superior to the use of air brackets during embryo loading (Abou-Setta, 2007). However, a better understanding of the transport of embryos within the uterine cavity will improve the protocols for embryo transfer procedures and increase the success rates of embryo implantation and pregnancies. The application of technology such as three- or four-dimensional sonography as well as focused research on the point of deposition of embryos will probably lead the way forward (Allahbadia *et al.*, 2002, 2005; Gergely *et al.*, 2005; Letterie, 2005).

References

Abou-Setta AM 2007 Air-fluid versus fluid-only models of embryo catheter loading: a systematic review and meta-analysis. *Reproductive BioMedicine Online* **14**, 80–84.

Allahbadia GN, Athavale UR, Gandhi GN *et al.* 2005 The final position of embryo-associated air at time of embryo transfer (ET) is unlikely to be a factor in predicting success in donor egg IVF recipient treatment cycles. *Fertility and Sterility* **84** (Suppl. 1), S270–S271.

Allahbadia G, Gandhi G, Athavale U *et al.* 2002 A blind embryo transfer is a rate limiting step to successful IVF. *Fertility and Sterility* **78** (Suppl. 1), S157–S158.

Anderson RE, Nugent NL, Gregg AT *et al.* 2002 Transvaginal

ultrasound-guided embryo transfer improves outcome in patients with previous failed in-vitro fertilization cycles. *Fertility and Sterility* **77**, 769–775.
Baba K, Ishihara O, Hayashi N *et al*. 2000 Where does the embryo implant after embryo transfer in humans? *Fertility and Sterility* **3**, 123–125.
Cavagna M, Contart P, Petersen CG *et al*. 2006 Implantation sites after embryo transfer into the central area of the uterine cavity. *Reproductive BioMedicine Online* **13**, 541–546.
Confino E, Zhang J, Risquez F 2007 Air bubble migration is a random event post embryo transfer. *Journal of Assisted Reproduction and Genetics* **24**, 223–226.
Coroleu B, Barri PN, Carreras O *et al*. 2006 Effect of using an echogenic catheter for ultrasound-guided embryo transfer in an IVF programme: a prospective, randomized, controlled study. *Human Reproduction* **7**, 1809–1815.
Eytan O, Elad D, Jaffa AJ 2007 Evaluation of the embryo transfer protocol by a laboratory model of the uterus. *Fertility and Sterility* **88**, 485–493.
Gergely RZ, DeUgarte CM, Danzer H *et al*. 2005 Three dimensional/four dimensional ultrasound-guided embryo transfer using the maximal implantation potential point. *Fertility and Sterility* **84**, 500–503.
Knutzen V, Stratton CJ, Sher G *et al*. 1992 Mock embryo transfer in early luteal phase, the cycle before in-vitro fertilization and embryo transfer: a descriptive study. *Fertility and Sterility* **57**, 156–162.
Krampl E, Zegermacher G, Eichler C *et al*. 1995 Air in the uterine cavity after embryo transfer. *Fertility and Sterility* **63**, 366–370.
Leong M, Leung C, Tucker M *et al*. 1986 Ultrasound-assisted embryo transfer. *Journal of In Vitro Fertilization and Embryo Transfer* **3**, 383–385.
Letterie GS 2005 Three-dimensional ultrasound-guided embryo transfer: a preliminary study. *American Journal of Obstetrics and Gynecology* **192**, 1983–1987; discussion 1987–1988.
Lewin A, Schenker JG, Avrech O *et al*. 1997 The role of uterine straightening by passive bladder distention before embryo transfer in IVF cycles. *Journal of Assisted Reproduction and Genetics* **14**, 32–34.
Marek DE, Langley MT, Pultorak MJ 2004 Incidence of retained embryos following embryo transfer when a full column of media is utilized to expel the embryos compared with a column of air. *Fertility and Sterility* **82** (Suppl. 2), S265.
Matorras R, Urquijo E, Mendoza R *et al*. 2002 Ultrasound-guided embryo transfer improves pregnancy rates and increases the frequency of easy transfers. *Human Reproduction* **17**, 1762–1766.
Nassar Z, Grossman M, Lakkis D *et al*. 2002 Lower implantation rate with downward displacement of ultrasonographic based echogenic drop following uterine embryo transfer. *Fertility and Sterility* **78** (Suppl. 1), S121.
Oliveira JB, Martins AM, Baruffi RL *et al*. 2004 Increased implantation and pregnancy rates obtained by placing the tip of the transfer catheter in the central area of the endometrial cavity. *Reproductive BioMedicine Online* **9**, 435–441.
Pacchiarotti A, Mohamed MA, Micara G *et al*. 2007 The impact of the depth of embryo replacement on IVF outcome. *Journal of Assisted Reproduction and Genetics* **24**, 189–193.
Pope CS, Cook EK, Arny M *et al*. 2004 Influence of embryo transfer depth on in vitro fertilization and embryo transfer outcomes. *Fertility and Sterility* **81**, 51–58.
Strickler RC, Christianson C, Crane JP *et al*. 1985 Ultrasound guidance for human embryo transfer. *Fertility and Sterility* **43**, 54–61.
Sundstrom P, Wramsby H, Persson PH, Liedholm P 1984 Filled bladder simplifies human embryo transfer. *British Journal of Obstetrics and Gynecology* **91**, 506–507.
Woolcott R, Stanger J 1998 Ultrasound tracking of the movement of embryo-associated air bubbles on standing after transfer. *Human Reproduction* **13**, 2107–2109.

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