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Ultrasound tracking of the movement of embryo-associated air bubbles on standing after transfer

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The aim of this study was to investigate whether standing upright shortly after embryo transfer has any potential to affect the position of embryos transferred to the uterine cavity during treatment with in-vitro fertilization (IVF). This was assessed by ultrasound-guided tracking of embryo-associated air within the uterine cavity. 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. No movement occurred 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) 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. 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.

Key words: embryo transfer/IVF/transvaginal ultrasound tracking

Introduction

There has been a steady decline in the time that patients have been requested to stay supine after embryo transfer. Clinicians and scientists who were involved with the provision of in-vitro fertilization (IVF) in the early 1980s will recall leaving patients in bed for prolonged periods following embryo transfer (Edwards *et al.*, 1980; Lopata *et al.*, 1980). Recommendations for patients to rest in bed for significant periods persist in more recent publications (Damewood, 1990; Gonen *et al.*, 1991). Fortunately, there has been a progressive increase in pregnancy rates, due to a wide range of scientific and clinical improvements. Nevertheless, this provides reassurance that standing shortly after embryo transfer is unlikely to be an important variable in determining pregnancy. Al-Shawaf *et al.* (1993) and Sharif *et al.* (1995) have both suggested that there is no justification for patients to remain supine after transfer, on the basis of excellent ongoing clinical pregnancy rates and high implantation rates. Botta and Grudzinskas (1997) could not demonstrate differences in pregnancy rate, miscarriage or implantation rate in a recent randomized prospective controlled study. None of these studies addressed the scientific basis for their proposition.

In this study, we have attempted to gain insight into whether standing upright shortly after transfer has any potential to influence the position of embryos within the uterine cavity. This has been assessed by using transvaginal ultrasound tracking of an air bubble injected into the uterine cavity along with the embryos. The methodology has clear limitations, in that it is not possible to track the embryos directly and assumes that the air bubble and embryos move in similar fashions. However, the absence of air bubble movement strongly implies that no movement of embryos occurs. Ultrasound tracking of embryo associated air bubbles is, in our view, the only available method of in-vivo observation of transfer associated intrauterine phenomena during an actual treatment cycle.

Materials and methods

A total of 101 consecutive embryo transfers on 93 patients was assessed during and after the procedure by transvaginal ultrasound. Of these patients, 44 underwent transfer of fresh embryos and in the remaining 57 patients, cryo-stored embryos were transferred. The stimulation regimen employed used luteal phase down-regulation with intra-nasal Nafarelin, followed by follicular phase Pergonal, Metrodin or Metrodin HP 150–450 IU (Serono, Sydney, Australia) together or in combination depending on the clinical circumstance of each patient. The transfer of fresh embryos was on day 2 post-egg collection. The transfer of cryopreserved embryos was in a natural menstrual cycle 4 days after documenting the commencement of the luteinizing hormone (LH) surge.

In all cases, a Jansen–Anderson K-JITS 2000 catheter set (Cook IVF, Brisbane, Queensland, Australia) was employed for embryo transfer which uses an outer guiding cannula and a soft 2 French transfer catheter, both of which are readily visible by transvaginal ultrasonography. A bivalve Graves speculum was placed into the vagina and the cervix was observed. Any excess cervical mucus was removed by wiping with a small gauze swab. The outer guiding cannula was passed through the cervix into a position where the operator believed it to be just within the endometrial cavity at the level of the internal cervical os. The speculum was then removed and then replaced by a 5 or 6 nHz transvaginal ultrasound probe to obtain a view of the outer guiding cannula and to ensure correct positioning. A satisfactory position of the cannula for embryo transfer was arbitrarily deemed to be central within the uterine cavity and 25–30 mm from the fundal surface of the endometrium. Notification to the embryologist was given and permission to load the embryos was obtained. The embryo transfer catheter was loaded with 5 µl of air proximal to 15–25 µl of medium [human tubular fluid (HTF);

Table I. Embryo associated air bubble movement on standing

Distance moved			
None	<1 cm	>4 cm	Uterine expulsion
95/101 (94.1)	4/101 (4.0)	2/101 (2.0)	0 (0)

Figures in parentheses represent percentages.

Irvine, CA, USA] and with 50% patient serum containing the embryos to be transferred. The catheter was then placed into the outer guiding cannula and its movement into the endometrial cavity was observed until the catheter was within 5–15 mm of the fundal endometrial surface. The embryos were then injected over 5–10 s, allowing observation of the movement of the transfer-associated air bubble into the uterine cavity. The air bubble was observed until movement ceased, while the embryologist checked the transfer catheter for unexpelled embryos. The ultrasound probe was then removed and the patient asked to stand immediately. The ultrasound probe was then reinserted into the vagina in a standing position and observation was made of the position and movement, if any, of the air bubble. The ultrasound probe was then removed and the patient was allowed to leave.

Results

It was technically easy to perform transvaginal pelvic ultrasound in an upright position and the procedure was readily accepted by patients. A clear view of the embryo transfer-associated air bubble was universally obtained. In no case was the bubble seen to move out of the endometrial cavity (Table I). In 5.9% (6/101) of transfers, movement was observed upon the patient adopting the standing position. In 4.0% (4/101) of transfers, <1 cm (range 2–5 mm), (three to cervix, one to fundus) movement of the embryo-associated air was observed on standing. In 2.0% (2/101) of transfers, movement of the air bubble by 4 cm and 5 cm respectively (towards the cervical canal) was seen. Extreme flexion (one anteflexion, one retroflexion) of the uterus was present in each of the two transfers with substantial movement. These were the only two patients in whom extreme flexion/version was apparent. The pregnancy rate for this series of embryo transfers was 27.7%. The average number of embryos transferred was 1.97, giving an implantation rate of 14.1%. Two of the four embryo transfers with air bubble movement of <1 cm resulted in pregnancy and neither of the two transfers with movement >1 cm led to pregnancy.

Discussion

Standing upright after embryo transfer is the first potential variable during the post-transfer phase of an IVF treatment cycle. It is impossible to assess what actually physically happens to embryos in this initial phase, or to assess their position or movement. Others have attempted to address related issues. Sharif *et al.* (1995) have published a non-randomized clinical experience of 103 cases in which the patient was asked to stand shortly after embryo transfer without apparent detriment to pregnancy rates. Al-Shawaf *et al.* (1993) attempted to address the issue of whether technical factors such as transfer catheter or ultrasound assistance had any impact on IVF treatment cycle outcome. In their study, patients were also asked to stand straight after transfer. The reported pregnancy and implantation rates in both studies remained high. 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 our understanding.

In our study, movement of the embryo transfer associated air bubble on standing was uncommon occurring in six of 101 transfers (5.9%). When movement did occur, it was usually extremely small (<5 mm), with the transfer associated air remaining in a favourable intrauterine position. We were able to demonstrate potentially important movement of the embryo transfer-associated air bubble in only two cases (2.0%). Where this occurred, movement of 4 cm and 5 cm respectively towards the uterine cervix was observed, but the air bubble still remained within the endometrial cavity. These patients were unique in being the only two patients in this series demonstrating extreme flexion and version of their uteri; one with extreme anteversion anteflexion, and the other with extreme retroversion retroflexion. Despite this, neither transfer was technically difficult.

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. Our laboratory experience 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. However, it is our opinion that no movement of the air bubble strongly implies that the embryos also remain stationary.

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. It needs to be appreciated that the so-called endometrial cavity is a potential and not a real space. Upon insertion of embryo transfer catheters the endometrial surfaces are separated and then re-oppose once the catheter is removed. The embryos, fluid and air injected into this potential space are then subjected to the contractile forces of the myometrium and endomyometrial

peristalsis (Birnholz, 1984; DeVries *et al.*, 1990). Indeed, we and others (Leyendecker *et al.*, 1996; Woolcott and Stanger, 1997) have recently demonstrated the considerable potential of endomyometrial peristalsis to have an effect on the outcomes of patients with infertility and particularly those being treated with IVF. It would seem likely that these forces along with surface tension generated by the fluid/solid interface are likely to be far more potent in determining the final position of transferred embryos.

Many patients in the past have expressed their concern about the possibility of embryos 'falling' out of the uterus on standing after their transfer. This is particularly so for those long term patients who had experienced previous requests to lie down for some time after embryo transfer. Progressively increasing pregnancy rates at the same time as reducing the length of time patients are allowed to lie down after embryo transfer gives us reassurance that standing up quickly is unlikely to be a factor which significantly affects pregnancy rates. Indeed the pregnancy (27.7%) and implantation rates (14.1%) in this series of embryo transfers compares favourably with the results of patients treated during the same period in our unit by non-ultrasound-guided embryo transfer who were not asked to stand immediately after transfer (pregnancy rate 18.5%, implantation rate 9.2%). It is appropriate, however, if only to answer our patients' concerns, that an attempt be made to demonstrate what movement, if any, of embryos might occur on standing. We believe the results of this study provide assurances to clinicians and patients alike that it is harmless to stand immediately after embryo transfer and that doing so is unlikely to be a significant factor affecting the position of embryos in the immediate post-transfer phase.

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