Human Reproduction vol.13 no.6 pp.1540–1546, 1998

Embryo transfer—can we learn anything new from the observation of junctional zone contractions?

P.Lesny[1,3,4], S.R.Killick[1,3], R.L.Tetlow[1], J.Robinson[2,3] and S.D.Maguiness[3]

[1]Academic Department of Obstetrics and Gynaecology, [2]Department of Biological Sciences, University of Hull, Cottingham Road and [3]The Hull IVF Unit, Princess Royal Hospital, Salthouse Road, Hull, UK

[4]To whom correspondence should be addressed at: The Hull IVF Unit, Princess Royal Hospital, Salthouse Road, Hull HU8 9HE, UK

To assess whether embryo transfer can alter junctional zone contractility, we studied the effect of easy and difficult mock transfers in 14 oocyte donors during in-vitro fertilization (IVF) cycles. An Echovist bolus (30 µl) was used to represent embryos and transfer medium. An 'easy' transfer was judged to be an atraumatic insertion of the catheter without touching the uterine fundus. A 'difficult' embryo transfer was mimicked by deliberately touching the uterine fundus twice with the soft end of the cannula. Transvaginal scan images were recorded, digitized and converted into five times normal speed to allow us to evaluate junctional zone contractility. Easy mock embryo transfers did not change endometrial mechanical activity. Echovist remained in the upper part of the uterine cavity and was not dispersed after 45 min. A difficult procedure generated strong random waves in the fundal area and waves from fundus to cervix which relocated the Echovist in six out of seven cases. We observed movements of the transfer bolus from the upper part of the uterus towards the cervix (four cases) and into Fallopian tubes (two patients). Our study confirms that the mechanical activity of the uterus is capable of relocating intrauterine embryos and that this activity depends on physical stimulation. Junctional zone contractions can be implicated in cases of IVF/embryo transfer failure or ectopic gestation.

Key words: embryo transfer/junctional zone contractions/transvaginal ultrasound

Introduction

The recognition of endometrial movements (Birnholz, 1984) has stimulated an area of research which may have significant implications for assisted reproduction. Recently, real time transvaginal ultrasound in connection with advanced audiovisual and computer technology have made more systematic investigations of endometrial activity possible.

Endometrial wavelike movements have been characterized (Ijland *et al.*, 1996, 1997b; Kunz and Leyendecker, 1996) and found to be of relevance to fecundability during the natural ovarian cycle (Ijland *et al.*, 1997a). Studies have also suggested that endometrial movements seem to affect sperm transport (Kunz *et al.*, 1996), and the pattern of contractions was changed in the presence of pelvic endometriosis (Leyendecker *et al.*, 1996). There is some evidence that a greater frequency of contractions on the day of embryo transfer appears to be associated with a reduced pregnancy rate (Fanchin *et al.*, 1997), but Woolcott and Stanger (1997) observed the converse.

Embryo transfer is one of the most critical steps affecting the success rate of in-vitro fertilization (IVF) and has changed little since IVF was first described 20 years ago (Steptoe and Edwards, 1978). The aim of transcervical embryo transfer is to manipulate a plastic catheter atraumatically through the cervix to the uterine cavity. While there is general agreement that a smooth embryo transfer is associated more frequently with a successful outcome (Wood *et al.*, 1985; Mansour *et al.*, 1990; Visser *et al.*, 1993), this opinion is not unanimous (Nabi *et al.*, 1997). However, clinical experience has shown that this procedure is far from perfect. Embryos have been found in the vagina following embryo transfer (Poindexter *et al.*, 1986; Schulman, 1986) and some embryo transfer techniques are more frequently associated with ectopic pregnancy (Yovich *et al.*, 1985). Clinical data from Waterstone *et al.* (1991) and Naaktgeboren *et al.* (1997) strongly suggest that the depth of the catheter placement is significant for pregnancy rate after IVF/embryo transfer. Experimental studies of mock embryo transfer showed expulsion of methylene blue in 57% of transfers (Mansour *et al.*, 1994) and movement of X-ray contrast medium towards the Fallopian tubes and cervix/vagina in 38.2 and 20.6% respectively (Knutzen *et al.*, 1992).

As endometrial mobility is minimal and progressively decreases during the luteal phase (Ijland *et al.*, 1996; Lesny *et al.*, 1997), interference with the endometrium at embryo transfer may change the contraction pattern and affect implantation in a mechanical way. We designed an observational study to test this theory in an environment as close as possible to a real IVF/embryo transfer cycle. To describe endometrial contractility, we use the term junctional zone contractions because there is evidence that this particular layer of myometrium, which consists of a discrete compartment of more compacted myocytes (Scoutt *et al.*, 1991; Tetlow *et al.*, 1997), may be responsible for the wavelike movements of the adjacent endometrium.

Materials and methods

Patients

This research project was approved by Hull and East Riding Research Ethics Committee; all patients were counselled and participated in it

on a voluntary basis after giving written consent. We asked 16 egg donors (multiparous women, age 23–33 years, mean 28.6) to act as model IVF/embryo transfer patients. None of them had any medical or gynaecological problems including previous history of gynaecological operation or Caesarean section. Patients were prospectively randomized into two groups to undergo a simulated easy or difficult mock embryo transfer.

Medication

Ovulation induction prior to IVF was achieved with a standard regimen of pituitary down-regulation with a luteinizing hormone releasing hormone superagonist (Nafarelin; Searle Pharmaceuticals, High Wycombe, UK) 800 μg daily administered from the mid-luteal phase, followed by appropriate doses of urofollitrophin (Metrodin High Purity; Serono Laboratories UK Ltd, Welwyn Garden City, UK). When the lead follicle reached a diameter of 20 mm, human chorionic gonadotrophin (Profasi, Serono) 10 000 IU, was given as an ovulatory trigger. Luteal support was provided by vaginal micronized progesterone (Utrogestan; Basins Iscovesco Laboratories, Paris, France) in a dose of 600 mg/night from the day of oocyte retrieval until the day of mock embryo transfer. All patients received 600 mg of ibuprofen (Brufen; Knoll Ltd, Nottingham, UK) 2 h before oocyte retrieval. Midazolam (Hypnovel; Roche Products, Welwyn Garden City, UK) was used for sedation and alfentanil (Rapifen; Janssen-Cilag Ltd, High Wycombe, UK) was given for analgesia during the procedure.

Imaging techniques

A transvaginal ultrasound scan (ATL Ultramark 4, 5 MHz transducer; Advanced Technology Laboratories, Seattle, USA) was performed for 5 min before and for 20 min after embryo transfer. Subsequently a 2–3 min ultrasound assessment was carried out every 5 min until a 45-min session was completed. At each examination scan images of mid-sagittal and transverse displays of the uterus were videotaped (VHS P4341; Goldstar, South Korea). After recording, the images were digitized into a computer equipped with a Perception Video Recorder 3500, PAL (Digital Processing System Inc. 1996, Scarborough, Canada) and converted to five times normal speed using Speed Razor Mach III (In: Synch Corporation, 1993, Bethesda, USA). A frame time-coding system allowed us to evaluate timing of events with an accuracy of ±0.04 s. A bolus of Echovist (Schering Health Care Ltd, Burgess Hill, Sussex, UK) was used to represent the embryo and transfer medium. Fine movements of the bolus were assessed by giving time-coded reference points. Contraction pattern and frequency were assessed and agreed by two observers. In those cases where movement of Echovist was present visualization was clear and timing by each observer was practically identical. We used the wave classification system introduced by Ijland *et al.* (1996), which includes five types of endometrial movements: no activity; waves from cervix to fundus; waves from fundus to cervix; opposing waves starting simultaneously at cervix and fundus; and random waves originating at various foci.

Embryo transfer

The patients were placed in a modified lithotomy position with an empty bladder. To facilitate an easy embryo transfer the shape and length of cervix and uterine cavity were assessed by ultrasound. For the mock embryo transfer we used an 'Embryon' catheter (Rocket Medical, Watford, UK) which had a soft inner catheter protruding from a more rigid outer sleeve. The catheter was loaded with 30 μl of Echovist organized in the same way as a transfer medium containing embryos during a real embryo transfer. The mock embryo transfer was performed 2 or 3 days after the oocyte retrieval. An easy embryo transfer was judged to be an atraumatic insertion of the inner soft end of the catheter without touching the uterine fundus. A difficult embryo transfer was imitated by deliberately touching the uterine fundus twice with the end of the inner catheter. In no case was the rigid outer sheath introduced as a guide or a tenaculum applied to the cervix. The Echovist bolus was expelled immediately by applying gentle pressure on a syringe plunger and the catheter was withdrawn a few seconds later. The tip of the catheter was examined for the presence of blood and retention of Echovist.

Table I. Junctional zone contraction waves observed immediately prior to embryo transfer at 2 and 3 days after oocyte retrieval

Type of waves	Random	Opposing	Cervico–fundal
Day 2 (8 patients)	5	5	3
Day 3 (6 patients)	6	2	0
Total	11	7	3

Results

Seven donors completed an IVF/embryo transfer cycle in both the easy and difficult transfer study groups. Recording just before embryo transfer revealed some endometrial activity in all patients. Seven women presented with more than one type of movement. Waves from cervix to fundus were seen in three out of 14 cases, but they were short and never involved the whole length of endometrium. Opposing waves were noted in seven out of 14 patients and random waves in 11 out of 14 patients. The direction of waves on days 2 and 3 after oocyte retrieval is shown in Table I. Endometrial activity on day 3 was less dynamic than on day 2, the waves being shorter and with limited spread. The mean frequency of movements before embryo transfer was 1.8 waves/min and was similar on day 2 and 3 but on day 3 random waves prevailed.

Easy embryo transfer was possible in all cases randomized to this study group. An atraumatic procedure did not alter endometrial mechanical activity. In all cases both the characteristics and frequency of waves remained the same as before embryo transfer. Mock embryos (Echovist) were seen as a split bolus (Figure 1A) in five patients and as a single bolus (Figure 1B) in two women. The division of Echovist was caused by its release from the catheter, later the contrast remained unmoved in all cases from the initial location in the upper part of the uterine cavity and was not dispersed even after 45 min. It also occupied a central position in the fundal area and did not move towards the cornua (Figure 2).

Stimulation caused by the 'difficult' embryo transfer had a dramatic effect on endometrial contractility. In all seven cases, we noted strong random waves, particularly intense in the fundal area, and waves directed from fundus to cervix which involved the full length of the endometrium. We also observed a higher frequency of contractions (mean 3.6 waves/min) in all cases. Knowing the distance Echovist had moved within the uterine cavity and the frame coded timing of these events, we were able to calculate the velocity for fundo–cervical and random waves as 2.04 mm/s and 0.68 mm/s respectively. We also noted very short contractions lasting 0.2 s which could move Echovist very rapidly inside the cavity and through the

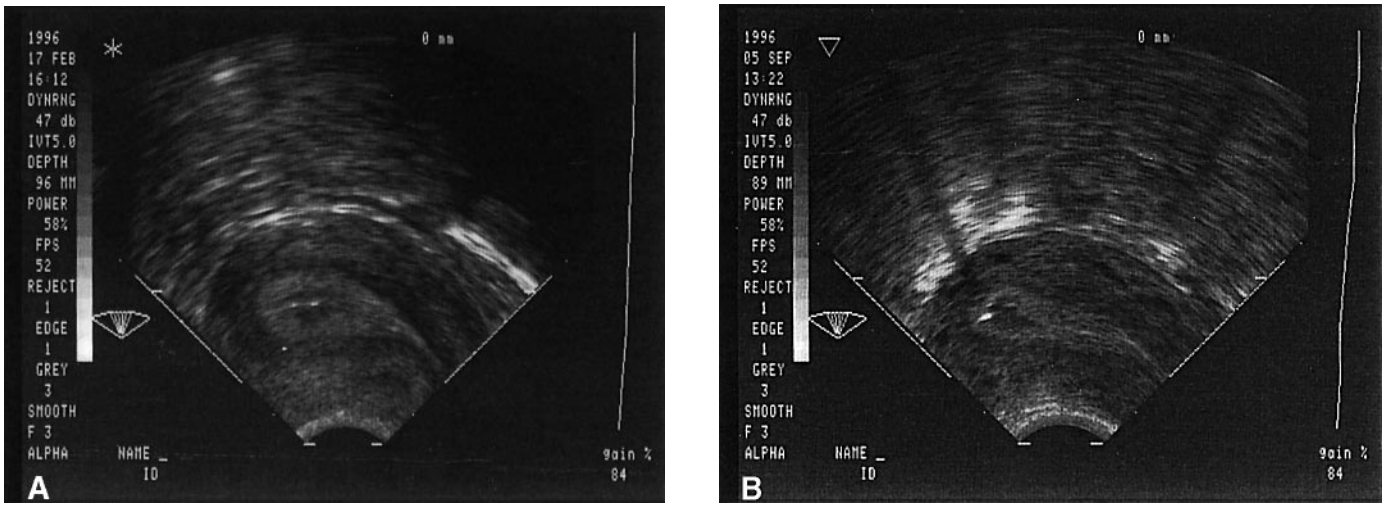

Figure 1. A transvaginal ultrasound scan of the uterus. Longitudinal section. Echovist after an easy mock embryo transfer remains in the upper part of the uterine cavity but presents two different types of images. (**A**) Divided bolus and (**B**) single bolus.

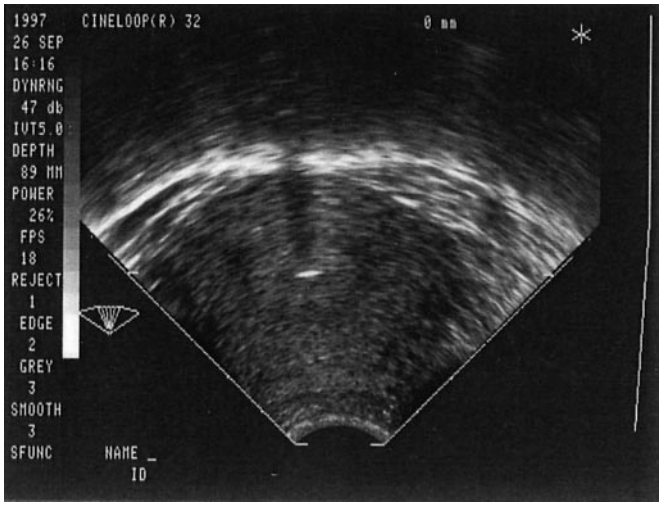

Figure 2. A transvaginal ultrasound scan of the uterus. Transverse section at the level of the uterine fundus. Forty-five minutes after an easy mock embryo transfer, Echovist still occupies a central position in the uterine cavity.

utero–tubal junction. The increased contractility was strong enough to disperse Echovist within 30 min and waves were still observed 45 min after embryo transfer. Mock embryos were relocated by contractions in six out of seven cases. We observed movement of the Echovist bolus from the upper part of the uterine cavity towards the cervix (Figure 3A, B, and C) and into the left and right Fallopian tubes (Figure 4A, B and Diagram 4A, B). Blood was seen on the end of the catheter in one out of seven and five out of seven cases of easy and difficult transfers respectively. There was no bleeding from the cervical canal on any occasion. The whole volume of Echovist was released from the cannula in all patients.

Discussion

These observations of the junctional zone contractions after mock embryo transfer provide information about the possible effects of transfer for treatment outcome. Our work with oocyte donors gave us a unique opportunity to evaluate embryo transfer in patients exposed to a long protocol regimen with down-regulation, superovulation and luteal phase support. Previous experimental studies of mock embryo transfer (Knutzen *et al.*, 1992; Mansour *et al.*, 1994) were undertaken in a natural cycle which has a vastly different hormonal environment. The dependence of junctional zone contractions on the phase of the ovarian cycle has been previously described (Oike *et al.*, 1988; De Vries *et al.*, 1990; Lyons *et al.*, 1991; Ijland *et al.*, 1996; Kunz and Leyendecker, 1996) and there is some evidence that junctional zone contractility could be more exaggerated in an IVF cycle than in a natural cycle (Abramowicz and Archer, 1990; Fukuda and Fukuda, 1994; Lesny *et al.*, 1997).

The use of Echovist has enabled us not only to assess the junctional zone contractions, but also to observe movements of the mock embryo *in utero*. Echovist is more stable and has better echogenicity than a simple air bubbled transfer medium which is necessary for adequate duration of observation. The physical characteristics of Echovist (Schlief *et al.*, 1993) are closer to a typical transfer medium than any other fluid used before (X-ray contrast media or methylene blue), but we do accept that they are not identical.

This study has demonstrated a connection between the ease of embryo transfer, endometrial wavelike movements and the mobility of mock embryos. An atraumatic mock embryo transfer did not have any effect on the junctional zone contractility and movement of Echovist. These results suggest that a difficult embryo transfer can be a critical factor limiting success rate as is known from clinical experience (Mansour *et al.*, 1990; Paulson *et al.*, 1990; Visser *et al.*, 1993; Sharif *et al.*, 1995). We tried to avoid touching the uterine fundus by using ultrasound measurement to identify the length of the uterus and then released Echovist 0.5–1 cm short of that distance. The placement of embryos in the mid-fundal area of the uterus has been found to be crucial for pregnancy rate (Rosenlund *et al.*, 1996). Waterstone *et al.* (1991) and Naaktgeboren *et al.* (1997) reported a significant rise in the pregnancy rate from 24 to 46% and from 17 to 37% respectively by changing only the depth of the catheter introduction to

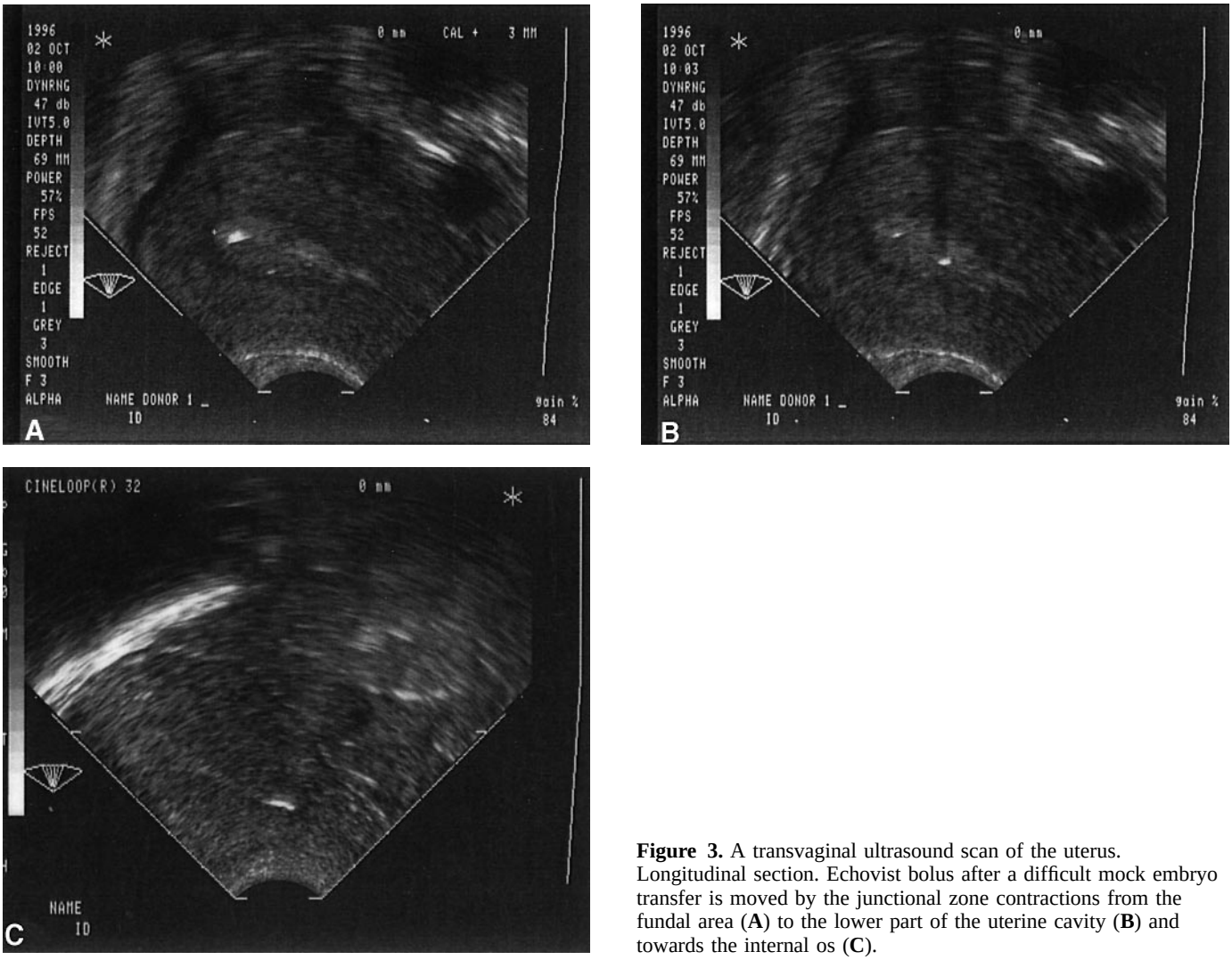

Figure 3. A transvaginal ultrasound scan of the uterus. Longitudinal section. Echovist bolus after a difficult mock embryo transfer is moved by the junctional zone contractions from the fundal area (**A**) to the lower part of the uterine cavity (**B**) and towards the internal os (**C**).

avoid placement of embryos close to the uterine fundus. We have demonstrated that stimulation of the uterine fundus affects junctional zone contractility. A small force applied twice by the soft end of the 'Embryon' catheter was sufficient to cause a turbulent uterine response. Fundo–cervical waves were generated and their velocity was even higher than that described by Ijland *et al.* (1997b) during the late follicular phase of natural cycles (peak of this activity). As endometrial waves of fundo–cervical direction moved Echovist towards the lower part of the uterus within 3–15 min, one could postulate that real embryos could have been expelled (Poindexter *et al.*, 1986; Schulman, 1986). Similar phenomenon was observed by Woolcott and Stanger (1997), where the embryo associated air bubble was rapidly moved towards the cervix during transvaginal ultrasound-guided embryo transfer.

Difficult embryo transfer also caused strong random waves in the fundal area which at first relocated mock embryos towards the cornua and then pushed them into the intramural segment of the Fallopian tube. Yovich *et al.* (1985) associated some embryo transfer techniques, usually involving higher cavity placement and possible trauma from the catheter, with an increased incidence of ectopic pregnancy. Our study has provided the first direct evidence that stimulation of the uterus during embryo transfer may be an aetiological factor in ectopic pregnancy by initiating the movement of embryos into the Fallopian tubes. It does not appear to be necessary to place embryos close to the tubal openings or release them rapidly from the catheter.

We chose fundal stimulation because in our opinion it was the most frequent type of stimulus unwillingly given to the uterus at embryo transfer and is not usually considered as a problem. A transfer catheter is more gentle than a uterine sound, a cervical dilator or a catheter with stylet which have all been claimed to have an adverse effect on the pregnancy rate (Wisanto *et al.*, 1989; Mansour *et al.*, 1990; Visser *et al.*, 1993). To our surprise, even this fine device was capable of generating evident contractility which relocated Echovist from an optimal position.

We saw blood on the catheter in five out of seven cases when we stimulated the uterine fundus and only once after easy embryo transfer which suggested that the endometrium was the most frequent source of bleeding. While some authors consider the presence of blood on the tip of the catheter or bleeding at the external os as a bad prognostic sign (Englert *et al.*, 1986; Ron-El *et al.*, 1994), others do not (Mansour *et al.*, 1990; Sharif *et al.*, 1995). If in real embryo transfer

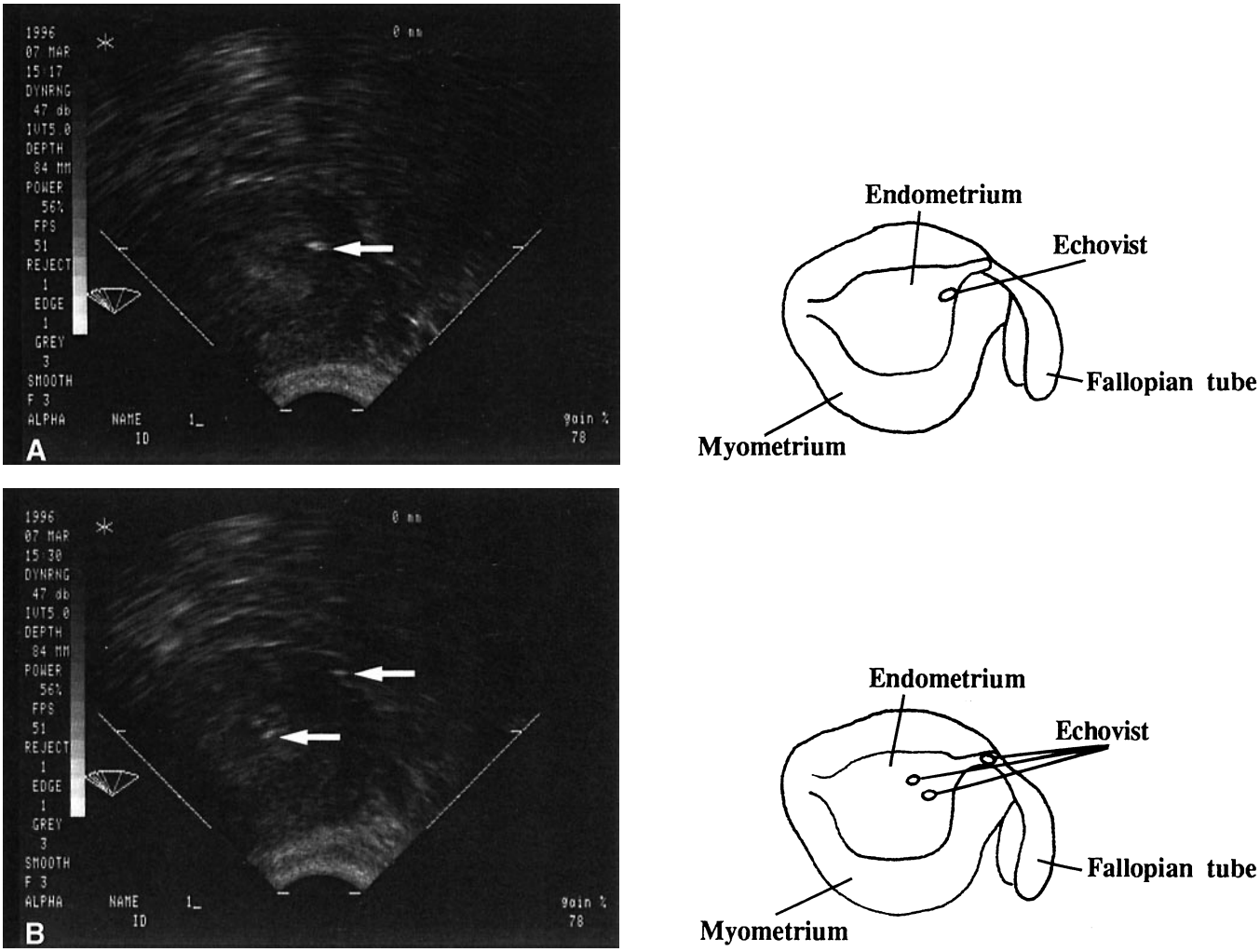

Figure 4. Transvaginal ultrasound scans and diagrammatic representations of the scan images. Transverse section at the level of the uterine fundus. Echovist bolus (arrows) after a difficult mock embryo transfer is moved by the junctional zone contractions towards the left cornual area (**A**) and pushed into the intramural section of the Fallopian tube (**B**).

contamination of the catheter is of endometrial origin (fundal area), contractions may follow and the pregnancy rate will be reduced. It will not be the case with a cervical source of bleeding, which might explain the lack of agreement in the scientific literature.

Our observation of the junctional zone contractions after mock embryo transfer suggests that there is no point in waiting for the release of embryos from the catheter or keeping the catheter '*in situ*' for 60 s after embryo transfer as recommended by Al-Shawaf *et al.* (1993) and Wisanto *et al.* (1989) and commonly practised by clinicians. We proved that an easy transfer does not stimulate any extra contractility. This is in agreement with a recent clinical study by Zech *et al.* (1997), who reported excellent results after quick atraumatic embryo transfer. We demonstrated that increased junctional zone contractility after difficult embryo transfer could be seen as late as 45 min after the procedure. In this situation, waiting 1 min for the uterus to settle would not change anything.

We did not notice any adverse effect of the dorsal position for embryo transfer in donors with anteverted uteri (all our patients). Knutzen *et al.* (1992) in the most frequently cited study about experimental embryo transfer described that after transfer in this 'unfavourable' position, radiographic contrast stayed '*in utero*' in only 48% of patients, whereas when the position was optimal 68% of women retained it. In our study, movement of Echovist appears to be secondary to junctional zone contractions and was not related to the uterine position or gravity. Knutzen *et al.* (1992) did not include difficulties with transfer in the evaluation of their results. In addition, every patient had the uterine cavity measured by sound prior to mock embryo transfer, which would have been a powerful stimulus of junctional zone contractions. Indeed, they later observed, without any image processing, uterine contractions in 47% of their patients. The relevance of the uterine position was questioned by Agarval *et al.* (1994). We think that it is not the uterine anatomy but difficulties with transfer due to anatomy followed by junctional zone contractility which is affecting experimental and clinical results.

Mansour *et al.* (1994) emphasized the importance of cervical mucus during embryo transfer. He found that methylene blue was extruded from the external os in 57% of cases when the mucus was not aspirated prior to mock embryo transfer compared to 23% when the mucus was removed. The rate of extrusion was also significantly smaller (22.5 and 77.5%

respectively) when a softer catheter was used (Wallace catheter; H.G.Wallace Ltd, Colchester, UK versus Craft catheter; Rocket of London, Watford, UK). We did not notice any movement of Echovist immediately following removal of the catheter after easy or difficult embryo transfer or within 45 min when an easy embryo transfer was concerned. However, the difficult embryo transfer usually resulted in relocation of the contrast within 5 min. This was similar to the timing of the methylene blue expulsion observed by Mansour *et al.* (1994). The above observation and better results achieved with the softer catheter may suggest that apart from cervical mucus, the embryo transfer technique may have had an important part to play in Mansour's study.

There is a difference in opinion as to whether increased baseline uterine activity at the time of embryo transfer is associated with increased (Woolcott and Stanger, 1997) or decreased (Fanchin *et al.*, 1997) receptivity. This may be the result of different methodologies and comparison of data should be made with caution.

In conclusion, our study confirms the existence of a mechanical force which can be one of the decisive factors for embryos to remain '*in utero*'. It shows a direct connection between the embryo transfer technique, uterine contractility and the mobility of embryos. The junctional zone contractions and endometrial wavelike movements of adjacent endometrium put embryo transfer into a new perspective by explaining several facts clinically felt for a long time. It also highlights a need for atraumatic transfer without touching the uterine fundus. The precise physiology and mechanism of these contractions are yet to be determined.

Acknowledgements

The authors wish to thank Dr Joanne Titterington, Mrs Susan Gray, Mrs Gillian Raven and all our IVF team for their assistance during this study. Mr Geoffrey Tomlinson and Mr Bruce Pears of the Audio–Visual Centre, the University of Hull are kindly thanked for editing film documentation and Miss Julie Olszewski for preparing this manuscript. We are grateful to all the egg donors who volunteered and made this project possible. Financial support for this research from The Hull IVF Trust and from Schering Health Care UK Ltd is gratefully acknowledged.

References

Abramowicz, J.S. and Archer, D.F. (1990) Uterine endometrial peristalsis – a transvaginal ultrasound study. *Fertil. Steril.*, **54,** 451–454.

Agarval, S.K., Coe, S. and Buyalos, R.P. (1994) The influence of uterine position on pregnancy rates with *in vitro* fertilization–embryo transfer. *J. Assist. Reprod. Genet.*, **11,** 323–324.

Al-Shawaf, T., Dave, R., Harper, J. *et al.* (1993) Transfer of embryos into the uterus: how much do technical factors affect pregnancy rates? *J. Assist. Reprod. Genet.*, **10,** 31–36.

Birnholz, J.C. (1984) Ultrasonic visualisation of endometrial movements. *Fertil. Steril.*, **41,** 157–158.

De Vries, K., Lyons, E.A., Ballard, G. *et al.* (1990) Contractions of the inner third of endometrium. *Am. J. Obstet. Gynecol.*, **162,** 678–682.

Englert, Y., Puissant, F., Camus, M. *et al.* (1986) Clinical study on embryo transfer after human *in vitro* fertilization. *J. In Vitro Fertil. Embryo Transfer*, **3,** 243–246.

Fanchin, R., Righini, C., Olivennes, F. and Frydman, R. (1997) Uterine contractions as visualised by ultrasound alter pregnancy rates in IVF and embryo transfer. *Hum. Reprod.*, **12,** 33–34.

Fukuda, M. and Fukuda, K. (1994) Uterine endometrial cavity movement and cervical mucus. *Hum. Reprod.*, **9,** 1013–1016.

Ijland, M.M., Evers, J.L.H., Dunselman, G.A.J. *et al.* (1996) Endometrial wavelike movements during the menstrual cycle. *Fertil. Steril.*, **65,** 746–749.

Ijland, M.M., Evers, J.L.H., Dunselman, G.A.J. *et al.* (1997a) Relation between endometrial wavelike activity and fecundability in spontaneous cycles. *Fertil. Steril.*, **67,** 492–495.

Ijland, M.M., Everes, J.L.H. and Hoogland, H.J. (1997b) Velocity of endometrial wavelike activity in spontaneous cycle. *Fertil. Steril.*, **68,** 72–75.

Knutzen, V., Stratton, C.J., Sher, G. *et al.* (1992) Mock embryo transfer in early luteal phase, the cycle before *in vitro* fertilization and embryo transfer: a descriptive study. *Fertil. Steril.*, **57,** 156–162.

Kunz, G. and Leyendecker, G. (1996) Uterine peristalsis throughout the menstrual cycle: physiological and pathophysiological aspects. *Hum. Reprod., Update*, **2,** No. 4, item 16, video, CD-ROM.

Kunz, G., Beil, D., Deininger, H. *et al.* (1996) The dynamics of rapid sperm transport through the female genital tract: evidence from vaginal sonography of uterine peristalsis and hysterosalpingoscintigraphy. *Hum. Reprod.*, **11,** 627–632.

Lesny, P., Tetlow, R., Robinson, J. *et al.* (1997) Uterine junctional zone contractions during IVF and embryo transfer cycle. *Hum. Reprod.*, **12,** 10–11.

Leyendecker, G., Kuntz, G., Wildt, L. *et al.* (1996) Uterine hyperperistalsis and dysperistalsis as dysfunction of the mechanism of rapid sperm transport in patients with endometriosis and infertility. *Hum. Reprod.*, **11,** 1542–1551.

Lyons, E.A., Taylor, P.J., Zengh, X.H. *et al.* (1991) Characterisation of subendometrial contractions throughout the menstrual cycle in normal fertile women. *Fertil. Steril.*, **55,** 771–774.

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

Mansour, R.T., Aboulghar. M.A., Serour, G.I. and Amin, Y.M. (1994) Dummy embryo transfer using methylene blue. *Hum. Reprod.*, **9,** 1257–1259.

Naaktgeboren, N., Broers, F.C., Heijnsbroek, I. *et al.* (1997) Hard to believe, hardly discussed, nevertheless very important for the IVF/ICSI results: embryo transfer technique can double or halve the pregnancy rate. *Hum. Reprod.*, **12,** 149.

Nabi, A., Awonuga, A., Birch, H. *et al.* (1997) Multiple attempts at embryo transfer: does this affect in-vitro fertilization treatment outcome? *Hum. Reprod.*, **12,** 1188–1190.

Oike, K., Obata, S., Tagaki, K. *et al.* (1988) Observations of endometrial movements with transvaginal sonography. *J. Ultrasound Med.*, **7,** 99.

Paulson, R.J., Sauer, M.V. and Lobo, R.A. (1990) Factors affecting embryo implantation after human in-vitro fertilization: an hypothesis. *Am. J. Obstet. Gynecol.*, **163,** 2020–2023.

Poindexter, A.N., Thompson, D.J., Gibbons, W.E. *et al.* (1986) Residual embryos in failed embryo transfer. *Fertil. Steril.*, **46,** 262–267.

Ron-El, R., Golan, A., Herman, A. *et al.* (1993) Assisted reproductive technologies. In Insler, V. and Lunenfeld, B. (eds), *Infertility. Male and Female*. Churchill Livingstone, Edinburgh, pp. 525–562.

Rosenlund, B., Sjoblom, P. and Hillensjo, T. (1996) Pregnancy outcome related to the site of embryo deposition in the uterus. *J. Assist. Reprod. Genet.*, **13,** 511–513.

Schlief, R., Schurman, R., Balzer, T. *et al.* (1993) Saccharide based contrast agents. In Nanda, N. and Schlief, R. (eds), *Advances in Echo Imaging Using Contrast Enhancement*. Kluwer Academic Publishers, Netherlands, pp. 71–96.

Schulman, J.D. (1986) Delayed expulsion of transfer fluid after IVF/embryo transfer. *Lancet*, **1,** 44.

Scoutt, L.M., Flyn, S.D., Luthringer, D.J. *et al.* (1991) Junctional zone of the uterus: correlation of MR imaging and histologic examination of hysterectomy specimens. *Radiology*, **179,** 403–407.

Sharif, K., Afnan, M. and Lenton, W. (1995) Mock embryo transfer with full bladder immediately before the real transfer for in-vitro fertilization treatment: the Birmingham experience of 113 cases. *Hum. Reprod.*, **10,** 1715–1718.

Steptoe, P.C. and Edwards, R. (1978) Birth after the reimplantation of a human embryo. *Lancet*, **ii,** 366.

Tetlow, R., Richmond, I., Greenman, J. *et al.* (1997) Morphometric analysis of the uterine junctional zone as seen by ultrasound. *Acta Obstet. Gynecol. Scand.*, **76,** 29.

Visser, D.S., Fourie, L.R. and Krugger, H.F. (1993) Multiple attempts at embryo transfer: effect on pregnancy outcome in an *in vitro* fertilisation and embryo transfer program. *J. Assist. Reprod. Genet.*, **10,** 37–43.

Waterstone, J., Curson, R. and Parsons, J. (1991) Embryo transfer to low uterine cavity. Lancet, **337,** 1413.

Wisanto, A., Janssens, R., Deschacht, J. *et al.* (1989) Performance of different embryo transfer catheters in human *in vitro* fertilization process. *Fertil. Steril.*, **52,** 79–84.

Wood, C., McMaster, R., Rennie, G. *et al.* (1985) Factors influencing pregnancy rates following *in vitro* fertilization and embryo transfer. *Fertil. Steril.*, **43,** 245–250.

Woolcot, R. and Stanger, J. (1997) Potentially important variables identified by transvaginal ultrasound-guided embryo transfer. *Hum. Reprod.*, **12,** 963–966.

Yovich, J.L., Turner, S.R. and Murphy, A.J. (1985) Embryo transfer technique as a cause of ectopic pregnancies in *in vitro* fertilisation. *Fertil. Steril.*, **44,** 318–321.

Zech, H., Stecher, A., Riedler, I. and Zech, A. (1987) High implantation rate with a fast and atraumatic embryo transfer technique. *Hum. Reprod.*, **12,** 156.

Received on December 1, 1997; accepted on March 23, 1998