Ultrasound-guided embryo transfer maximizes the IVF results on day 3 and day 4 embryo transfer but has no impact on day 5

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BACKGROUND: The use of ultrasound-guided embryo transfer has been reported to affect success rates in some centres but not others. In a prospective study, we examined the influence of ultrasound guidance in embryo transfer performed on different days after oocyte retrieval. METHODS: Two different methods of embryo transfer were evaluated in 1069 consecutive transfers. The ultrasound-guided embryo transfer was used in 433 cases, whereas 636 embryo transfers were performed with the tactile assessment (‘clinical feel’) method. RESULTS: Ultrasound-guided embryo transfer yielded a higher overall pregnancy rate than the ‘clinical feel’ approach, 47 versus 36% (P < 0.001). This difference was statistically significant where embryos were transferred after 3 or 4 days of culture, 45.9 versus 37.1% (P = 0.001) and 42.3 versus 27% (P = 0.035) respectively but not significant (P = 0.112) on day 5 embryo transfer (56.3 versus 45.7%). Likewise, the implantation rate was significantly different between the two groups on day 3 and 4 embryo transfer, 23.3 versus 15.8% (P < 0.01) and 21.6 versus 15.7% (P < 0.05%) respectively but no statistical difference was noted on day 5 embryo transfer, 26.7 versus 23.6%. CONCLUSION: Ultrasound assistance in embryo transfer on day 3 and 4 significantly improved pregnancy rates in IVF but had no impact on day 5.

Key words: embryo transfer/ultrasound-guided embryo transfer/IVF/uterine receptivity/window of implantation

Introduction

The success of IVF is dependent on each step of the procedure. Improvements in ovulation induction, oocyte retrieval and laboratory techniques [including intracytoplasmic sperm injection (ICSI)] have achieved maximum fertilization rates and embryo development and ~90% of all cases where oocytes have been retrieved in an IVF programme have at least one embryo transferred per cycle. Implantation failure is a major factor limiting the success of IVF. On average, up to 90% of apparently healthy zygotes transferred in utero are destined to vanish, giving no signs of trophoblastic attachment and production of human chorionic gonadotrophin (HCG) (Nikas et al., 1999).

Although implantation is poorly understood in humans, the pregnancy rate following embryo transfer is influenced by several defined variables. Identified variables in embryo transfer include embryo quality (Scott et al., 1991), number of attempts and presence of blood or mucus on the transfer catheter (Nabi et al., 1997), age of the patient, number of embryos transferred, cause of infertility (Roseboom, 1995), and the presence of uterine contractions (Fanchin et al., 1998). Furthermore, catheter choice (Gonen et al., 1991) or use of ultrasound-guided transfer (Prapas et al., 1995; Woolcot and Stanger, 1997; Coroleu et al., 2000; Wood et al., 2000) have been reported to affect success rates in some centres, but not others (Al Shawaf et al., 1993; Nabi et al., 1997; Kan et al., 1999). Some studies report that difficulty of embryo transfer is not detrimental to pregnancy outcome (Tur-Kaspa et al., 1998), others that difficult transfers and the provider performing the embryo transfer may negatively affect the success rate (Leeton et al., 1982; Nabi et al., 1997; Hearns-Stokes et al., 2000), raising concern that ultrasound-guided embryo transfer may be a major determinant in a successful transfer. Furthermore, it was suggested that during the embryo transfer, movements for location of catheter tip in normal transfer catheters may lead to endometrial trauma (Woolcot and Stanger 1997; Letterie et al., 1999).

In the present study, two different transfer methods have been evaluated in terms of pregnancy rate. A prospective study was initiated to evaluate how ultrasound-guided embryo
transfer can influence the success rate of IVF in relation to the day of embryo transfer.

Materials and methods
This study was based on a 19 month survey of two different embryo transfer procedures: ultrasound-guided or tactile assessment (‘clinical feel’) embryo transfer in our IVF programme. Data were prospectively collected for 1069 consecutive embryo transfer procedures on 917 women in the Iakentro Assisted Reproduction Treatment Programme from January 1999 to July 2000. Transfers of cryopreserved embryos were not included. Admission of a patient to the assisted reproduction programme depended on strict criteria. In general, patients >42 years of age and those with FSH concentrations >15 IU/ml on cycle day 3 were excluded from the study. Pituitary down-regulation was achieved by administering the gonadotrophin-releasing hormone agonist triptorelin (0.1 mg s.c.) in a long stimulation protocol (Prapas et al., 1995). Multiple follicle development was induced with 225–300 IU/day of recombinant human FSH (Gonal-R; Serono Pharmaceuticals, Switzerland) or highly purified urinary FSH (Metrodin; Serono Pharmaceuticals). The criterion for using recombinant FSH or not was the patient’s insurance financial coverage. A single injection of 10 000 IU of human chorionic gonadotrophin (Profasi; Serono Pharmaceuticals) was administered to induce the final stage of oocyte maturation, and transvaginal, ultrasound-guided follicular aspiration was performed 34–36 h later.

Study design
Included in the study were cases with at least one good quality embryo in relation to the day of embryo transfer. Classification of embryos was based on either the number of blastomeres or the developmental stage. In determining which embryos were suitable for day 3 transfer, parameters such as fragmentation and equality of blastomeres were taken into consideration, whereas for day 4 and day 5 transfer, compaction, cavitation and expansion were taken into consideration (Veeck, 1998). Embryo transfer after 3 days of culture usually occurred at the 6–8-cell stage of embryonic development. Embryos that did not reach the 6-cell stage after 3 days of culture were considered retarded. Four days after oocyte retrieval, morulae and cavitating morulae were observed. Embryos at earlier stages were classified as retarded. After 5 days of culture, blastocysts and other embryo stages were observed. Non-cavitating embryos were considered retarded. For statistical reasons we quantified the scoring system of Veeck (1998) as follows. On day 3, embryos presenting 6–7 cells were given 1 point, 8–10 cells 2 points, and embryos in compaction process 3 points. Additionally we added 3 points for the A quality embryos, 2 points for the B and 1 point for the C. On day 4 embryos were given 3 points for young blastocyst, 2 points for morulae of A quality and 1 point for morulae of B quality. For day 5 embryos, morulae were given 1 point, young blastocysts 2 points, blastocysts 3 points and expanded blastocysts 4 points.

Between one and four embryos that demonstrated the most advanced developmental stage and best morphology were selected for transfer. Women of ≤36 years old had a maximum of three embryos transferred while up to four embryos were transferred in cases of women >36 years old. All cases in which transfer of retarded embryos occurred were excluded from the study.

Progesterone supplementation was given, beginning on the day of embryo retrieval. The laboratory techniques and the culture media have been described previously (Vanderzwalmen et al., 1996; Gardner et al., 1998).

Embryo replacement was carried out in two different gynaecological rooms 3–5 days after oocyte retrieval. All women were told to come in on the day with a relatively full bladder but were not told whether or not they would have their embryo transfers done under ultrasound guidance. The choice of the embryo transfer procedure was based upon which rooms were available for the transfer. The women that were going to have their embryo transfer in the first room, where the ultrasound machine was, had been advised to keep their bladder full in order to provide an acoustic window to visualize the uterine cavity. The women that were placed for embryo transfer in the second room, where ultrasound was not available, had no advice. All patients were placed in the lithotomy position without any anaesthesia or sedation.

The number of embryos replaced depended upon the age of the patient, the number of embryos available for replacement, and the number of previous IVF attempts.

After the insertion of a bivalve speculum to expose the cervix, the exocervix was cleaned of cervical mucus using a cotton swab and a small amount of culture medium. Concurrently, in the adjacent embryo culture laboratory, the morphological appearance of the embryos was evaluated for the last time and the best embryos were selected and loaded into an embryo Wallace catheter (Medical Supplies, England) for embryo transfer. Embryo quality was estimated using the scoring system of Veeck (Veeck, 1998). During this period a trans-abdominal ultrasound was performed, using the convex probe (3.5 MHz) of a real time ultrasound (General Electric RT-X 200) to evaluate the length of the endometrial cavity, the angle between the internal os and the cavity and to determine the site of the thickest endometrial appearance. Once the transfer catheter was loaded, the embryologist presented it to the gynaecologist performing the replacement procedure. The Wallace catheter used for the procedure consisted of an outer teflon guiding sleeve through which the inner catheter, which is an open–ended silicone catheter with 1.6 mm external diameter, where the embryos are loaded, could be advanced. The tip of the catheter’s outer sleeve was angled appropriately for anteverted or retroverted uteri and after being inserted to the cervical canal was gently advanced to the internal orifice under transabdominal ultrasound control. Once the tip of the catheter reached the internal orifice the inner embryo transfer catheter was advanced through the canal to the uterine cavity and addressed to the thickest part of the endometrial appearance, where the embryos were deposited utilizing 20–25 µl of fluid. After a period of 60 s, the catheter was carefully removed and it was ascertained under the microscope that the embryos had been washed out of the catheter. Subsequently, the speculum was removed, the bladder was discharged, the gynaecological table transformed to a normal bed and the women were left resting for 60 min. For the women who had their embryo transfer in the second room, where the ultrasound machine was not available, the embryo transfer was still performed using the same Wallace catheter by the same gynaecologist without ultrasound control as previously described (Diedrich et al., 1989).

Statistical analysis
One-way ANOVA was used in order to detect any statistically significant differences between the two groups (‘ultrasound guidance’ and ‘clinical feel’) with respect to the number of embryos transferred, the mean quality of embryos (quantified using the scoring system of Veeck), the mean number of oocytes retrieved and fertilized, and the age of the subject, on embryo transfer days 3, 4 and 5 respectively. P < 0.05 was considered statistically significant.

Fisher’s exact test was used to test for differences between pregnancy rates in the ‘ultrasound guidance’ and ‘clinical feel’ groups.
Results

The mean age of patients, and number and quality of embryos replaced in the two groups are summarized in Table I. The mean number of embryo numbers transferred and the embryo quality were similar in both groups, as assessed by the scoring system (Veeck, 1998). The distributions of other indications (oocytes retrieved and fertilized) were not significantly different between the compared groups.

In all, 435 pregnancies were achieved corresponding to a pregnancy rate of 40.5% per embryo transfer procedure. Embryo transfer on day 3 achieved 220 pregnancies (38.19%), on day 4 gave 64 pregnancies (34.97%) and on day 5 gave 151 pregnancies (48.7%). The ultrasound-guided procedure yielded a significantly higher overall pregnancy rate than the ‘clinical feel’ procedure for day 3 \((P = 0.001)\) and day 4 embryo transfer \((P = 0.035)\) but no significantly difference in pregnancy rate was noted for day 5 embryo transfer (Table II). Likewise, the implantation rate was significantly different between the two groups for day 3 and 4 embryo transfer \((P < 0.01\) and \(P < 0.05\) respectively) but no statistical difference was noted for day 5 embryo transfer (Table II). The mean number of embryos transferred per attempt was not different between the two groups, nor was the quality (Table I).

Discussion

The embryo transfer procedure is typically performed blindly and seems to contribute to the high failure rate of IVF. As a result, correct timing, stage of embryo development, number and quality of embryos, receptivity of the uterus and instrumentation have been investigated by different groups, but no agreement exists as to the importance of each of these variables in the success of IVF. The relative influence of the embryo transfer technique and the catheter used for transfer is still debatable. It has been shown that an easy atraumatic transfer is essential for successful implantation (Leeton et al., 1982; Wood et al., 1985; Englert et al., 1986; Diedrich et al., 1989).

Strickler et al. proposed the use of ultrasound guidance for transvaginal transcervical human embryo transfer (Strickler et al., 1985); this study showed that the embryo transfer was facilitated with ultrasound guidance. In addition, ultrasound-guided embryo transfers were rated easier and there was less catheter distortion, in comparison to embryo transfers guided by ‘clinical feel’.

Wisanto et al. compared different embryo transfer catheters in an IVF programme in a prospective and randomized study on 400 consecutive embryo transfers (Wisanto et al., 1989). One of the groups had the embryo transfer using the TDT catheter under ultrasound guidance. They concluded that the TDT catheter was the easiest to use but a significantly lower pregnancy rate was obtained compared with the other catheters; an improvement of the pregnancy rate was achieved when the TDT catheter was used under ultrasound guidance.

In both the aforementioned studies the catheters used for embryo transfer under ultrasound control consisted of a guiding catheter which first was introduced to the uterine fundus, then...
pulled back 1 cm and the inner catheter for embryo transfer pushed forward.

A subsequent study (Hurley et al., 1991) investigated the use of transvaginal ultrasound guidance in embryo transfer, but did not identify a significant difference between this and the usual clinical feel method. However, it must be taken into account that ultrasound guidance was used only after clinical insertion of the catheter. Later, in a prospective study using abdominal ultrasound (Al-Shawaf et al., 1993), it was found that there was no significant effect of ultrasound on pregnancy outcome.

Woolcott and Stanger studied 121 consecutive transvaginal ultrasound-guided embryo transfers (Woolcott and Stanger, 1997). Observation was made of guiding cannula and transfer catheter placement in relation to the endometrial surface and uterine fundus during embryo transfer. They concluded that tactile assessment of embryo transfer catheter placement was unreliable since, in 17.4% of transfers, the outer guiding catheter inadvertently abutted the fundal endometrium, the outer guiding cannula indented the endometrium in 24.8% and the transfer catheter embedded in the endometrium in 33.1%. Unavoidable sub-endometrial transfers occurred in 22.3% and avoided accidental tubal transfer in 7.4%.

In our opinion, the above procedures had multiple movements made with the catheter already placed in the uterine cavity. This increases the risk of uterine contraction and embryo expulsion (Fanchin et al., 1998; Lesny et al., 1998). In addition, irritating the endometrial epithelium to start decidualization is probable (De Feo, 1963).

These reports prompted us to perform our own comparative study using either the ‘ultrasound guided’ or the ‘clinical feel’ embryo transfer procedure. The only prerequisite was that the transfers should be done by the same gynaecologist who was specialized in prenatal diagnosis, in cordoncentesis and other difficult ultrasound-guided procedures, and who had the ability to drive the transfer catheter into the uterine cavity with the fewest possible movements. The advantages of the method were: (i) it was sonographically evident that the outer sleeve of the Wallace catheter did not enter the uterine cavity and the inner catheter was driven to the thickest area of the endometrium; (ii) the fluid bubbles containing the embryos were seen coming out of the catheter into the endometrial cavity; (iii) in all cases the full bladder straightened the uterine–cervical axis and the catheter insertion was atraumatic.

Our data have shown that the ultrasound-guided procedure yielded a significantly higher overall pregnancy rate than the ‘clinical feel’ procedure on embryos transferred 3 or 4 after retrieval, but no statistically significant difference in pregnancy rate was noted for embryos transferred after 5 days. Another potentially interesting observation is that the level of statistical significance between the two procedures declined from the third to the fourth day of embryo transfer, becoming insignificant after day 5. However, it should be noted that the imbalance of patients in the two embryo transfer groups after 5 days of embryo culture (n = 87 for the ultrasound group, versus n = 223 for the ‘clinical feel’ group) may, at least in part, have contributed to this observed lack of statistical significance.

The term ‘uterine receptivity’ or ‘window of implantation’ was introduced in animal models to define the short time lapse during which the uterus allows oocyte implantation to occur (Psychoyos, 1976, 1986; Yoshinaga, 1988; Denker, 1994). In humans there is evidence that this period lasts for only 24–40 h (Martel et al., 1981, 1989; Nikas et al., 1995) and is characterized by the presence of pinopodes. The use of antiprogestins in rats and rabbits has shown that the window of implantation can be postponed or advanced according to the progesterone treatment (Sarantis et al., 1988; Beier et al., 1994). The short phase of ‘uterine receptivity’ is progesterone dependent (Prapas et al., 1998) and is preceded by a prereceptive neutral endometrial state followed by a refractory state of non-receptivity (Psychoyos, 1976, 1986; Psychoyos and Prapas, 1987). Embryo transfer during the neutral phase may result in implantation. The neutral and the receptive phases combined comprise the ‘window of embryo transfer’. However, once the endometrium has entered the receptive period it is impossible to prevent progression of maturation to the refractory period (Psychoyos and Prapas, 1987; Sarantis et al., 1988). It has been suggested that the appearance of endometrial pinopodes signifies the end of the window for embryo transfer (Psychoyos and Martel, 1990) unless embryos are transferred at the blastocyst stage (Bolton, 1994). In animals, it has been shown that mechanical trauma of the endometrium or other endometrial stimuli (liquids, hormonal replacement) acting on a sensitive uterus (day 4 post ovulation) will provoke endometrial decidualization (De Feo, 1963). It has also been postulated that mechanical trauma in some cases can provoke a small decidua formation of the endometrium acting on days 3 or 5 after ovulation (De Feo, 1963). Once endometrial sensitivity has been acquired, the endometrium loses its capacity for another response to stimuli (De Feo, 1963).

Based on the data cited above, it seems that the endometrial trauma provoked during the embryo transfer, in some cases, could alter the endometrial maturation and the uterine contractions. Our data show that endometrial trauma could have negative effects on day 3 and 4 embryo transfer due to the provoked asynchrony between embryo stage and endometrial phase; however, it has no effect on day 5 embryo transfer where the embryos are at blastocyst stage and no asynchrony can be provoked. Obviously, any factor related to endometrial trauma during the embryo transfer (physician’s experience, type of embryo catheter, full bladder, difficult transfer etc.) can influence the pregnancy rates when embryo transfers are performed on day 3 or 4 but has no effect on day 5 of embryo transfer.

Embryo transfer is the last decisive step on the way to a pregnancy following human IVF. Further development of the embryo following the transfer into the uterus is presently beyond our control. Since implantation is a poorly understood phenomenon in humans, it is of clinical value to exploit the apparent improvement in the potential implantation rate. Although it is difficult to consider the influences on a potential pregnancy at the time of embryo transfer, our data suggest a significantly better performance of the ultrasound-guided procedure in relation to the efficiency of establishing pregnancy. Therefore we recommend the use of ultrasound with day 3 and 4 embryo transfer as first choice in an IVF programme.
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Acknowledgements
The authors thank David Olive M.D. and Ali Utku M.D. for their helpful advice during the preparation of the manuscript.

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Received on March 13, 2001; accepted on June 14, 2001