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The usefulness of ultrasound guidance in frozen–thawed embryo transfer: a prospective randomized clinical trial

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BACKGROUND: Recent randomized controlled trials have shown that implantation and pregnancy rates were improved with ultrasound-guided embryo transfer compared with clinical touch in fresh IVF cycles associated with supraphysiological ovarian steroid levels. However, the usefulness of ultrasound guidance in frozen–thawed embryo transfer where potential hormonal influences are lacking has not been appropriately investigated. METHODS: A total of 184 consecutive patients undergoing thawed embryo transfer cycles with hormone replacement under pituitary suppression were randomized by computer-generated randomization table to two study groups: 93 had ultrasound-guided (group 1) and 91 had clinical touch (group 2) embryo transfer. RESULTS: There was equal distribution between the two study groups with respect to the main demographic and baseline characteristics of the patients as well as the characteristics of both prior IVF cycles from which embryos were generated and cryopreserved–thawed embryo transfer cycles. However, both pregnancy and implantation rates in group 1 (34.4 and 19.8% respectively) were significantly higher than the corresponding values (19.7 and 11.9%) in group 2. CONCLUSIONS: Ultrasound guidance in frozen–thawed embryo transfer significantly increases pregnancy and implantation rates.

Key words: embryo transfer/frozen–thawed embryos/implantation rates/IVF/ultrasound-guided embryo transfer

Introduction

Traditionally, embryo transfer has been viewed as an unimportant variable in the success of an IVF treatment cycle. However, the need to revisit embryo transfer technique has recently been stressed (Kovacs, 1999; Salha *et al*., 2001) and at present, embryo transfer is the focus of particular attention as an essential factor associated with IVF failure or success (Schoolcraft *et al*., 2001; Pasqualini and Quintans, 2002). Various facets of embryo transfer practice may be pivotal in improving implantation rates, but avoiding excessive contractions and trauma to the endometrium are considered to be principal factors associated with optimal implantation rates after transcervical embryo transfer (Schoolcraft *et al*., 2001). In this regard, it is noteworthy that recent randomized controlled trials by us (Coroleu *et al*., 2000) and others (Tang *et al*., 2001) have reported that implantation and pregnancy rates were improved with ultrasound-guided embryo transfer compared with clinical touch.

Improved success rates using ultrasound-guided embryo transfer may be related, at least in part, to the problem of uterine contractions. Uterine junctional zone contractions or endometrial wavelike movements have been reported in both natural and IVF cycles (Ijland *et al*., 1996; Lesny *et al*., 1998a; Eytan *et al*., 2001). Increased uterine contractility assessed by ultrasonography at the time of embryo transfer has been shown to be associated with reduced pregnancy and implantation rates in IVF cycles and, importantly, a direct relationship between the embryo transfer technique, uterine contractility and the relocation of embryos towards the cervix has been reported (Fanchin *et al*., 1998; Lesny *et al*., 1998b, 1999a,b). Thus, atraumatic embryo transfers, with a soft catheter and without touching the uterine fundus, which have no effect on uterine junctional zone contractility and the replaced embryos, would improve pregnancy and implantation rates (Lesny *et al*., 1999a,b). Ultrasonographic guidance at the time of embryo transfer may have several potential advantages in this regard, such as facilitating the placement of soft catheters and avoiding contact with the uterine fundus and endometrial disruption (Schoolcraft *et al*., 2001).

The above notwithstanding, mechanical activity of the uterus at the time of embryo transfer depends not only on physical stimulation but also on hormonal influences (Fanchin *et al*., 1998, 2000; De Ziegler *et al*., 2001; Eytan *et al*., 2001). Thus,

it has been shown that uterine contractility throughout the IVF cycle is more exaggerated than in spontaneous menstrual cycles (Lesny *et al*., 1998a; Epiney *et al*., 2000). We have previously reported that ultrasound assistance in fresh embryo transfer significantly improved pregnancy and implantation rates in IVF cycles associated with supraphysiological ovarian steroid levels (Coroleu *et al*., 2000). We report here the results of a prospective investigation undertaken to ascertain whether ultrasound guidance could also improve pregnancy rates in transfer of frozen–thawed embryos in artificially prepared cycles where estrogen and progesterone profiles mimic the natural cycle.

Materials and methods

Patients studied, design, and sample size

We included in the present study 184 consecutive patients undergoing thawed embryo transfer cycles. All subjects were IVF patients who had their own cryopreserved pre-embryos thawed at the IVF programme of the Institut Universitari Dexeus' Reproductive Medicine Service. Oocyte donation cycles were excluded. Women were aged 30–44 years and the main patient indications for IVF/ICSI included male factor, tubal infertility, unexplained infertility, and endometriosis. On the day of embryo transfer, but prior to the procedure being carried out, these patients were randomly assigned, according to a computer-generated randomization table, to two study groups: 93 had ultrasound-guided (group 1) and 91 had clinical touch (group 2) embryo transfer.

The sample size was calculated after considering our previous study comparing ultrasound guidance versus clinical touch for fresh embryo transfer after IVF (Coroleu *et al*., 2000), where we reported a 16% difference in clinical pregnancy rates in favour of the ultrasound group. Assuming a power of 80% to detect such a difference between groups in the pregnancy rates obtained with a type I risk of 0.05, $\leq$90 women per group were needed.

IVF and cryopreservation protocols

Protocols for ovarian stimulation and luteal phase support used in our assisted reproduction programme have been reported in detail elsewhere (Coroleu *et al*., 2000, 2002; Martinez *et al*., 2000). Briefly, multiple follicular development for IVF and fresh embryo transfer is routinely accomplished using gonadotrophin treatment with FSH under pituitary suppression with leuprolide acetate. In those patients with a previous poor response and/or basal (cycle day 2–4) FSH serum concentrations $>$10.5 IU/l which, according to our experience, is associated with poor response cycles (Barri *et al*., 2000), we use a flare-up protocol with leuprolide acetate and ovarian stimulation is accomplished with FSH in combination with hMG. The ovulatory injection of hCG was administered when a consistent rise in serum estradiol concentrations was associated with the presence of two or more follicles $>$18 mm in diameter. Oocyte aspiration was performed by vaginal ultrasonography 35–37 h after hCG injection. IVF procedures, including ICSI, used in our programme have also been previously described in detail elsewhere (Calderón *et al*., 1995).

Embryos were cryopreserved 48 or 72 h after oocyte retrieval at the 4–8-cell stage. This was accomplished according to a previously reported protocol (Veiga *et al*., 1987), where 1,2-propanediol and sucrose solution in phosphate-buffered saline (PBS) are used as cryoprotectants (Testart *et al*., 1986). Only embryos having $\geq$4 cells on day 2 or $\geq$6 cells on day 3 and $<$30% fragmentation were eligible for freezing. Thawing was performed as reported elsewhere (Testart *et al*., 1986; Veiga *et al*., 1987). Embryos surviving the freezing procedure ($\geq$50% of their initial number of blastomeres intact) were cultured for 24 h and cleaved embryos were preferentially replaced.

Embryo transfer protocol

Patients received programmed cycle endometrial preparation with previous pituitary suppression with a GnRH agonist. A single i.m. injection of 3.75 mg of depot triptorelin (Decapeptyl 3.75 mg; Ipsen Pharma S.A., Barcelona, Spain) was given on days 20–22 of the menstrual cycle. At the onset of menses or 14–16 days following the injection and after a hypogonadotrophic state (estradiol serum levels $<$50 pg/ml) was evidenced, endometrial stimulation with estrogen was initiated. The standard estrogen therapy was estradiol valerate (Progynova; Schering España S.A., Madrid, Spain) 8 mg/day in three divided doses for 13 days. On day 14, 200 mg vaginal micronized progesterone, three times daily (Utrogestan; Laboratorio Seid, Barcelona, Spain), was given concomitantly. Estradiol and progesterone serum levels were measured on day 15 for luteal phase evaluation.

Uterine transfer of thawed embryos was carried out on the fourth day of progesterone treatment. One to three embryos per patient were replaced depending upon the age of the patient, the number of previous embryo transfers, and the number and quality of embryos available for replacement. Embryo quality was established according to the number and form of blastomeres and the percentage of cytoplasmic fragmentation, as previously suggested (Plachot and Mandelbaum, 1990). For statistical comparison purposes, and in order to quantify the embryo quality objectively, those three variables were coded according to fixed criteria and then assigned an arbitrary score of 0, 2 or 4 as previously reported (Coroleu *et al*., 2002). Embryos having $<$4 blastomeres on day 2, or $<$6 blastomeres on day 3, after IVF, were scored 0. In contrast, $\geq$4- and $\geq$6-cell embryos on days 2 and 3, respectively, were scored 2. Irrespective of the day after IVF, symmetrical cells were scored 4 whereas asymmetrical cells scored 0. Embryos having $<$15%, 15–49.9% and $\geq$50% fragmentation, were scored 4, 2 and 0 respectively, both on days 2 and 3. Accordingly, an optimal quality embryo would score 10. For the final analysis of results, the embryo score per patient was considered as the mean value of the scores given to each of the transferred embryos.

The preparation for embryo transfer was the same for both study groups (Coroleu *et al*., 2000, 2002). Patients were placed in the lithotomy position and the cervix was exposed using a bivalve speculum. The exocervix was cleaned with a phosphate-buffered saline (PBS) solution (Dulbecco's PBS solution; Irvine Scientific, Santa Anna, CA, USA) and the endocervical mucus was removed by means of a sterile Teflon catheter (Malleable Stylet Wallace, Simcare, Lancing, West Sussex, UK) connected to a syringe.

The Edwards–Wallace embryo replacement catheter (SIMS Portex Ltd, Kent, UK) connected to an insulin syringe was used for all embryo transfers. This is a soft silicon catheter possessing a stiffer outer sheath that stabilizes the softer inner cannula, which carries the embryos and actually enters the endometrial cavity for embryo transfers. The catheter was first loaded with transfer medium [50% synthetic serum substitue (Irvine Scientific) and IVF-50 medium (Scandinavian IVF Science, Göthenburg, Sweden)], taking care to avoid air bubbles. The embryos were loaded in the catheter.

The catheter was handed to the clinician who inserted it through the cervical canal. It was at this point that a difference in technique was introduced between the two groups. In the ultrasound-guided group both the insertion and the positioning of the catheter were facilitated by transabdominal (with full bladder) ultrasonography [Tosbee (SSA-240A), convex 3.75 MHz transducer; Toshiba Co., Tokyo, Japan]. The embryo(s) were slowly released when the ultrasound scan showed the catheter to be 15–20 mm from the fundus

endometrial surface, a depth of embryo replacement which, according to our previous study (Coroleu *et al*., 2002), is associated with increased implantation rates. In the clinical touch group, the embryo(s) were released according to the clinician's feeling as to the position of the catheter (i.e. advancing the catheter tip into the uterus to a point located at 15–20 mm from the fundus according to the ultrasonographic measurement of the uterine cavity performed within 3 months of embryo transfer).

In all transfers, only 30 µl of transfer medium containing the embryos were gently expelled into the uterine cavity. The catheter was gently removed immediately after transfer and then checked under a stereomicroscope to ensure that all embryos had been transferred. At the end of the procedure, patients remained resting in bed for 30 min. Difficult embryo transfer was defined as a procedure involving one or more of the following: cervical resistance leading to a prolonged procedure (>5 min), the need to use increased force or cervical grasping, the need for cervical dilation, the presence of blood on the catheter, or multiple (two or more) sequential attempts because of embryos being retained in the catheter system.

Patients were advised to continue with estrogen and progesterone treatment and were tested for β-hCG serum concentration 14–16 days following embryo transfer. If pregnancy was confirmed, hormonal treatment was maintained until 10 weeks of gestation. Clinical pregnancy was diagnosed by increasing serum concentrations of β-hCG and the subsequent demonstration of an intrauterine gestational sac by ultrasonography. Only clinical pregnancies were considered for the evaluation of results.

Hormone analysis

Serum estradiol, progesterone and β-hCG were determined by electrochemiluminescence immunoassay (ECLIA) on the Elecsys 1010 analyser (Roche Diagnostics, Basel, Switzerland). The inter- and intra-assay coefficients of variation were 6.2 and 5.7% for estradiol, 5.4 and 2.4% for progesterone, and 5.1 and 4.5% for β-hCG respectively.

Statistical analysis

Data were analysed by the Statistical Package for Social Sciences (SPSS, Chicago, IL, USA). We used the χ^2-test to compare qualitative variables, and Student's *t*-test to compare quantitative variables. The significance level was set at $P < 0.05$.

Results

The results are summarized in Tables I–IV. There was equal distribution between the two study groups with respect to the main demographic and baseline characteristics of the patients, including age, body mass index, cause and duration of infertility, FSH concentration in the early follicular phase, and previous assisted reproduction techniques performance (Table I). Table II summarizes the data regarding the prior fresh IVF treatment cycles from which embryos were generated for cryopreservation. Type of stimulation protocol, ovarian response as evaluated by estradiol levels and number of oocytes retrieved, the number of patients with ICSI, fertilization rate, the number of embryos suitable for replacement and cryopreservation, and clinical pregnancy rates obtained after fresh embryo transfer were similar for the two study groups.

Characteristics of the cryopreserved–thawed embryo transfer cycle in groups 1 and 2 are presented in Table III. No differences between groups were found regarding the total number of embryos thawed, the day of embryo freezing, the time of embryo storage, and the embryo survival rate. It should be noted that both the number of embryos per replacement and the mean embryo score per replacement were very similar in the two groups studied. Hormonal levels associated with estrogen and progesterone treatment, as well as the endometrial response to this therapy, were also similar in groups 1 and 2. The number of embryo transfers considered difficult was higher, albeit not significantly so, in group 2 as compared with group 1 (Table III). The number of patients with blood-stained catheters at the end of embryo transfer and the number of patients with retained embryos requiring a repeated transfer tended to be less in group 1 (two and two respectively) than in group 2 (six and five respectively), though this was not statistically significant. Interestingly, however, both clinical pregnancy and implantation rates were significantly higher in the group of patients undergoing embryo transfer under ultrasonographic guidance (group 1) (Table IV). There were no ectopic pregnancies.

Table I. Main demographic and baseline characteristics and previous assisted reproduction technique performance of patients in group 1 (ultrasound-guided) and group 2 (clinical touch)

Variable[a]	Group 1 (n = 93)	Group 2 (n = 91)
Age (years)[b]	36.6 ± 3.4	36.2 ± 3.0
Body mass index (kg/m^2)[b]	22.1 ± 2.0	22.4 ± 1.9
Cause of infertility (n, %)		
Male	46 (49.5)	36 (39.6)
Tubal	25 (26.8)	29 (31.8)
Unexplained	20 (21.5)	23 (25.3)
Endometriosis	2 (2.2)	3 (3.3)
Duration of infertility (years)[b]	6.0 ± 2.3	5.6 ± 1.8
Day 2–4 FSH (IU/l)[b]	6.7 ± 1.8	7.0 ± 2.1
No. of previous fresh embryo transfers	113	110
Total no. embryos replaced	306	298
No. of embryos/replacement[b]	2.7 ± 0.4	2.7 ± 0.5
No. of previous frozen–thawed embryo transfers	21	23
Total no. of embryos replaced	52	61
No. of embryos/replacement[b]	2.3 ± 0.8	2.3 ± 0.7

[a]No significant differences between groups.
[b]Values are means ± SD.

Table II. Characteristics of IVF cycles from which embryos were generated for cryopreserved–thawed embryo transfers in group 1 (ultrasound-guided) and group 2 (clinical touch)

Variable[a]	Group 1 (n = 93)	Group 2 (n = 91)
Stimulation protocol (n, %)		
Long	73 (78.5)	76 (83.5)
Flare-up	20 (21.5)	15 (16.5)
Estradiol (pg/ml) on HCG day[b]	2164 ± 787	2098 ± 775
No. of oocytes retrieved[b]	13.2 ± 2.5	13.3 ± 2.5
No. of patients with ICSI (n, %)	57 (61.2)	53 (58.2)
Fertilization rate (%)[b]	72.5 ± 10.5	73 ± 9.9
No. of embryos/patient[b]	7.8 ± 1.6	8.2 ± 1.6
Clinical pregnancies (n, %)	17 (18.3)	14 (15.4)

[a]No significant differences between groups.
[b]Values are means ± SD.

Table III. Cryopreserved–thawed embryo transfer cycle characteristics in group 1 (ultrasound-guided) and group 2 (clinical touch)

Variable[a]	Group 1 (n = 93)	Group 2 (n = 91)
No. of embryos thawed	276	294
Day of embryo freezing (n, %)		
Day 2	14 (15)	10 (11)
Day 3	79 (85)	81 (89)
Time of cryopreservation (months)[b]	5.6 ± 4.6	6.1 ± 5.2
Embryos survived (n, %)	219 (79.3)	234 (79.6)
Embryos transferred (n, %)	199 (90.9)	204 (87.2)
No. of embryos/replacement[b]	2.1 ± 0.8	2.2 ± 0.9
Mean embryo score/replacement[b]	7.0 ± 1.4	7.4 ± 1.2
Endometrial thickness (mm) on the day of embryo transfer[b]	11.4 ± 2.3	11.2 ± 2.0
Estradiol (pg/ml) (2 days before transfer)[b]	149 ± 49	155 ± 41
Progesterone (ng/ml) (2 days before transfer)[b]	9.5 ± 2.4	9.7 ± 3.0
Difficult embryo transfer (n, %)	6 (6.5)	11 (12.1)

[a]No significant differences between groups.
[b]Values are means ± SD.

Table IV. Implantation and pregnancy rates and outcome of gestation in group 1 (ultrasound-guided) and group 2 (clinical touch)

Variable[a]	Group 1 (n = 93)	Group 2 (n = 91)
Clinical pregnancies/embryo transfer	32 (34.4)[b]	18 (19.8)[b]
Single	26 (81.2)	12 (66.6)
Twins	6 (18.7)	6 (33.3)
Implantation rate (%)	19.1[c]	11.7[c]
Spontaneous miscarriage	7 (21.9)	4 (22.2)

[a]Values are n (%).
[b,c]Values in rows with common superscripts were significantly different ($P < 0.05$).

It should be noted that most patients undergoing frozen embryo transfer were those who did not become pregnant with their fresh embryo transfer cycle. This may explain the clinical pregnancy rates in fresh IVF cycles reported in Table II for both control and study groups which were lower than frozen–thawed embryo transfer pregnancy rates reported in Table III.

Discussion

Frozen–thawed embryo transfer aims to increase the possibilities for conception in addition to those obtained through the prior fresh IVF and embryo transfer cycle. It is also a useful tool for lowering the risks of both ovarian hyperstimulation syndrome and multiple conception by allowing the cancellation of the fresh transfer. However, the implantation rate of frozen–thawed embryos is usually lower than that obtained with fresh transferred embryos (Mandelbaum, 2000). A recent study (Wang *et al*., 2001) analysing the influence of clinical factors on implantation rates in frozen–thawed embryo transfer cycles concluded that female age, the cause of infertility and the outcome of prior fresh embryo transfer are the most important success factors for implantation following frozen embryo transfer. However, technical aspects of embryo transfer were not considered in that study.

Interestingly, there is now a growing body of evidence indicating that the technique of embryo transfer, as traditionally performed, is inadequate and may contribute to decreased IVF efficiency and success. In fact, although embryo transfer appears deceptively simple, various facets of the transfer procedure may be pivotal in improving implantation rates (Kovacs, 1999; Salha *et al*., 2001; Schoolcraft *et al*., 2001; Pasqualini and Quintans, 2002). We and others have recently shown that ultrasonographic guidance is an essential factor associated with successful embryo transfer and implantation after fresh IVF cycles (Coroleu *et al*., 2000; Tang *et al*., 2001). However, the potential usefulness of ultrasonography in the process of frozen–thawed embryo transfer has not been appropriately investigated.

Tang *et al*. recently reported a randomized controlled trial comparing embryo transfer under ultrasound guidance (n = 400) versus the clinical touch method (n = 400) (Tang *et al*., 2001). A total of 441 fresh cycles and 359 frozen–thawed cycles were included in that study. The overall implantation rate was significantly higher in the ultrasound-guided group. There was no significant improvement in the pregnancy rate, but a consistent trend towards a better pregnancy rate in the ultrasound group in both fresh and frozen–thawed cycles was observed (Tang *et al*., 2001). In that study, however, frozen–thawed embryos were replaced in natural cycles, clomiphene citrate-induced cycles or controlled cycles using pituitary down-regulation followed by hormone replacement therapy. Importantly, a dummy transfer was performed immediately before the real transfer (Tang *et al*., 2001). A mock embryo transfer may generate uterine contractility (Lesny *et al*., 1998b, 1999b) and precludes ultrasound-guided embryo transfer from its main advantages, i.e. not touching the endometrium and the uterine fundus with replacement of the embryos in the lumen of the endometrial cavity, which are considered to be the most important factors for successful embryo transfer by most IVF teams (Kovacs, 1999; Salha *et al*., 2001).

The present investigation provides further data favouring the use of ultrasound-guided embryo transfer for improving pregnancy rates and has several original features in this respect. Firstly, the two groups of patients studied were similar with respect to the clinical factors which influence the implantation rate in frozen–thawed embryo transfer cycles (Wang *et al*., 2001), as discussed above. As with patient and fresh IVF cycle characteristics (Tables I and II), characteristics of the cryopreserved–thawed embryo transfer cycles (Table III) were also similar in groups 1 and 2, thus adding to the validity of our results. Secondly, a mock embryo transfer was not performed in our study. Thirdly, as in our previous study (Coroleu *et al*., 2002), all transfers were carried out by the same person, thus avoiding any impact of the 'physician factor' on implantation rates (Karande *et al*., 1999; Hearns-Stokes *et al*., 2000), and using the most popular embryo transfer catheter (Wood *et al*., 2000; Salha *et al*., 2001) according to a strictly standardized protocol. Finally, all embryo transfers were performed in artificially prepared cycles with prior pituitary suppression.

This latter point seems important given that controlled ovarian hyperstimulation has been reported to have an adverse

effect on the uterine environment and conception outcome (Check *et al*., 1995, 1999). Rapid uterine contractions have been observed immediately after oocyte retrieval, which were not noted immediately prior to follicular puncture (Lesny *et al*., 1998a). Thus, it has been suggested that there is a release of prostaglandins and/or other inflammatory reaction mediators from the multifollicular/multiluteal ovaries (Lesny *et al*., 2002). This effect would persist into the luteal phase and, together with supraphysiological concentrations of serum estradiol and only brief exposure to progesterone, could be responsible for more exaggerated contractions in an IVF cycle compared with natural cycles (Lesny *et al*., 2002).

For patients included in the present study there was no deleterious effect of hormonal influences associated with controlled ovarian hyperstimulation, and thus any potential increase in mechanical activity of the uterus at the time of embryo transfer was dependent only on physical stimulation. Futhermore, ultrasound-guided embryo transfer may have two additional advantages over clinical touch embryo transfer when considering that: (i) blind catheter placement has been shown to result in a malposition of the catheter in >25% of cases, thus indicating that tactile assessment of embryo transfer catheter position is unreliable (Woolcott and Stanger, 1997); and (ii) the depth of the embryo replacement into the uterine cavity influences implantation rates, with highest pregnancy rates obtained when embryos are replaced 15–20 mm from the fundus endometrial surface (Coroleu *et al*., 2002).

All the above supports the notion that ultrasound assistance in embryo transfer is a pivotal tool for improving pregnancy rates in assisted reproduction irrespective of whether embryos are fresh or frozen and replaced in spontaneous, stimulated or artificially prepared cycles. A very recent report showing that ultrasound-guided embryo transfer improves outcome in patients with previous failed IVF cycles (Anderson *et al*., 2002) provides further evidence in this regard.

In conclusion, ultrasound guidance in frozen–thawed embryo transfer significantly increases pregnancy and implantation rates.

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