Day 3 embryo transfer with combined evaluation at the pronuclear and cleavage stages compares favourably with day 5 blastocyst transfer

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BACKGROUND: The respective advantages of day 3 and day 5 embryo transfer are a matter of debate. Previous comparisons did not include pronuclear stage zygote scoring and cumulative success rates (fresh and cryopreserved embryos). METHODS: Patients were randomized prospectively for day 3 or day 5 embryo transfer. Day 3 embryos were selected for transfer and cryopreservation by using combined evaluation at the pronuclear and cleavage stages. RESULTS: There was no difference between day 3 and day 5 fresh embryo transfers as to the rates of pregnancy (58 versus 62%), clinical pregnancy (56 versus 58%), delivery (50 versus 48%), implantation (35 versus 38%) and birth (33 versus 36%) rates. The corresponding values for cryopreserved embryo transfers were also similar. However, day 3 embryo transfer compared favourably with day 5 transfer when the pregnancy (90 versus 66%), clinical pregnancy (85 versus 62%) and delivery (77 versus 52%) rates were calculated per oocyte recovery attempt. CONCLUSIONS: With a selected population of good prognosis patients and our embryo selection criteria, the implantation potential of day 3 and day 5 embryos is equal. Per oocyte recovery attempt, day 3 transfer is more clinically efficient than day 5 transfer, but at least one transfer of cryopreserved embryos is necessary to manifest this superiority.

Key words: blastocyst transfer/embryo quality evaluation/embryo transfer policy/pronuclear scoring

Introduction
The commercialization of sequential culture media capable of supporting in-vitro development of human embryos to the blastocyst stage has encouraged many centres to change the embryo transfer policy and to postpone embryo transfer to day 5 after fertilization instead of the conventional day 2 or 3 transfer (Gardner et al., 2000a; review). Higher implantation rates after fresh (Marek et al., 1999; Hsieh et al., 2000; Milki et al., 2000; Schoolcraft and Gardner, 2000; Langley et al., 2001) and cryopreserved (Langley et al., 2001) embryo transfers have been reported when the transfer was performed on day 5 as compared with days 2 or 3. A high implantation rate is a prerequisite for reducing the number of embryos to be transferred at a time which, in its turn, will reduce the risk of multiple pregnancies. Thus, the claimed increase in the implantation rate, after the switch to the day 5 embryo transfer policy, was welcome as an important step in this direction (Scholtes and Zeilmaker, 1996; Marek et al., 1999; Huismans et al., 2000; Gardner et al., 2000b; Milki et al., 2000; Schoolcraft and Gardner, 2000; Vidaeff et al., 2000).

However, other studies have reported comparable pregnancy (Scholtes and Zeilmaker, 1996; Coskun et al., 2000; Huismans et al., 2000; Hsieh et al., 2000) and implantation (Coskun et al., 2000; Huismans et al., 2000) rates after day 3 and day 5 embryo transfers. It is also known that, with the current techniques, most embryos do not become blastocysts during extended culture, and it is not clear how many of these embryos would have implanted if replaced at the cleavage stage (Alper et al., 2001). In view of these conflicting data, the question of the overall benefit of blastocyst culture and transfer needs to be revisited.

The use of different criteria for the selection of embryos for transfer may be at least partly responsible for the discrepancies between different studies comparing day 3 and day 5 embryo transfer outcomes. In the past few years the possibilities of viable embryo selection at the early cleavage stages have been improved substantially by introducing non-invasive scoring criteria applicable as early as the pronuclear stage (Scott and Smith, 1998; Tesarik and Greco, 1999) and by refining the scoring criteria for cleaving embryos (Gerris et al., 1999; Van Royen et al., 1999, 2001). This prospective randomized study reports pregnancy and implantation rates achieved with the use of combined pronuclear and cleavage-stage evaluation criteria and day 3 embryo transfer as compared with those
achieved with day 5 blastocyst transfer. These comparisons are made for fresh embryo transfers only, for cryopreserved embryo transfers only and for cumulative outcomes from fresh and cryopreserved embryo transfers.

Materials and methods

Study design and patient selection
This study was restricted to couples with female age of <38 years who were treated by ICSI and who had ≥8 two-pronucleated zygotes on the day following ICSI. All patients selected were randomized on the day following oocyte retrieval by a computer-generated randomization list either to day 3 or day 5 transfer.

Assisted reproduction techniques
Controlled ovarian hyperstimulation was performed with the use of recombinant human FSH (Puregon; Organon, Oss, The Netherlands) after pituitary suppression with buserelin acetate (Suprefact; Suprefact Hoechst; Marion Roussel, Milan, Italy) started in the late luteal phase of the previous cycle as described (Ubaldi et al., 1999). Ovulation was induced by 10 000 IU HCG (Profasi; Serono, Rome, Italy) when at least three follicles had reached a diameter of ≥18 mm, and transvaginal follicle aspiration was performed, under ultrasound guidance, 36 h later (Ubaldi et al., 1999).

Oocytes were freed from the cumulus oophorus by a brief incubation (15–30 s) at 37°C in 40 IU/l hyaluronidase solution (Hyase; Vitrolife, Gothenburg, Sweden), followed by mechanical removal of the corona radiata with the use of finely drawn denuding pipettes (SAGE BioPharma, Bedminster, NJ, USA) and subjected to ICSI using previously described techniques and instruments (Rienzi et al., 1998). Fertilization was assessed by three sequential inspections of the sperm-injected oocytes performed between 12 and 16 h after ICSI. Only those oocytes that showed two pronuclei and two polar bodies during at least one of these inspections were considered further for eventual embryo transfer. Abnormally fertilized oocytes (1 or 3 pronuclei) were excluded from further consideration.

Normally fertilized oocytes (zygotes) were cultured in G1.2 medium up to day 3 after ICSI and in G.2.2 medium (both media purchased from Vitrolife) from day 3 to day 5 where applicable. The cultures were carried out at 37°C, and the media were equilibrated with 5% CO₂ in air. Two best-scoring embryos, selected with the criteria described below, were transferred to the patient’s uterus on either day 3 or day 5 according to the study design. When the pronuclear and cleavage-stage scores were not in agreement for day 3 transfers, the pronuclear score was given priority. For day 5 transfers, blastocyst morphology was given priority to pronuclear score in case of discrepancy. The remaining good quality embryos were cryopreserved if they did not show a developmental blockage (no developmental change throughout the last 24 h of culture). Day 3 and day 5 embryo cryopreservation was performed with freeze-kit 1 and freeze-kit 2 (both purchased from Vitrolife) respectively, according to the manufacturer’s instructions.

Zygote and embryo quality evaluation
Zygote quality was evaluated during three observations performed between 12 and 16 h after ICSI with the use of previously described criteria (Tesarik and Greco, 1999). Zygotes were considered morphologically normal when at least 3 nucleolar precursor bodies (NPB) were present in each pronuclei; when the difference in number of NPB between the two pronuclei did not exceed 3; and when the NPB were similarly distributed (random or polarized) in both pronuclei. Zygotes showing abnormal pronuclear pattern were assigned to a single group as reported (Tesarik et al., 2000).

Cleaving embryos were evaluated on days 2 and 3 after ICSI with the use of a cumulative embryo classification scheme taking into account cleavage speed, blastomere symmetry, extent of fragmentation, and the presence or absence of multinucleated blastomeres (Table I). Those embryos that received the lowest number of points in each cohort were considered to have the highest implantation potential.

Blastocyst evaluation
Blastocyst evaluation was performed after 5 days of in-vitro culture. The blastocysts were considered available for transfer or cryopreservation when a big blastocoele was present (at least half the volume of the embryo), when the inner cell mass was identifiable and when the trophoderm was formed by many cells forming a cohesive epithelium.

Statistical analysis
Significance of differences in the success rates between the day 3 and day 5 transfer protocols was evaluated by χ²-test using StatView II statistical package (Abacus Concepts, Berkeley, California, USA).

Results
For the purposes of this study the success rates characterizing assisted reproduction outcomes were defined as follows. Pregnancy rate and clinical pregnancy rate were calculated as the number of attempts with positive βHCG (>20 IU/l) 2 weeks after ICSI and with the detection of embryonic heartbeat on ultrasound at 8 weeks gestation respectively, divided by the number of embryo transfer procedures. Delivery rate was obtained by dividing the number of deliveries by the number of embryo transfer procedures. Ongoing pregnancies are not reported because this study was concluded only after all clinical pregnancies had gone to term or resulted in a spontaneous abortion. Implantation rate was calculated by dividing the number of embryonic sacs in intrauterine position by the number of embryos transferred. Birth rate was obtained by dividing the number of babies born by the number of embryos transferred. These success rates were calculated separately for fresh and cryopreserved embryo transfers. Cumulative pregnancy, clinical pregnancy, delivery, implantation and birth rates from both fresh and cryopreserved embryo transfers were calculated per embryo transfer. In addition, cumulative pregnancy, clinical pregnancy and delivery rates from fresh and cryopreserved embryo transfers were also calculated per oocyte recovery attempt.

The basic characteristics of the patient were similar between the two groups (day 3 and day 5 embryo transfer): mean age ± SD (31.6 ± 3.1 versus 32.2 ± 2.5; not significant), mean ± SD oocytes retrieved (12.7 ± 7.1 versus 13.1 ± 5.2; not significant), fertilization rate (71.2 versus 71.8%; not
significant) and cleavage rate (92.8 versus 91.3%; not significant).

Two cleaving embryos and two blastocysts were transferred to each patient of the day 3 and day 5 group respectively. The blastocyst formation rate per fertilized oocyte obtained in the day 5 embryo transfer group was 44.8% (211/470). Cryopreservation was performed for those patients for whom supernumerary good quality embryos or blastocysts were available. This occurred more frequently in the day 3 group (42/48 patients) as compared with the day 5 group (18/50 patients; \( P < 0.01 \)).

When the two presumably best embryos were being chosen from each patient’s embryo cohort for day 3 transfer, the pronuclear score agreed with the day 3 score in 216 out of 258 embryos evaluated (84%) and disagreed in the remaining 42 (16%) embryos.

The comparison of success rates between day 3 and day 5 transfer did not show any difference for either fresh (Table II) or cryopreserved (Table III) embryos. When cumulative success rates from fresh and cryopreserved embryo transfers were calculated per embryo transfer, there was no difference between the day 3 and day 5 transfers as to the pregnancy rate, clinical pregnancy rate and delivery rate, but the implantation and birth rates were slightly higher (\( P < 0.05 \)) for the day 5 transfer (Table IV). On the other hand, cumulative pregnancy, clinical pregnancy and delivery rates were substantially higher for the day 3 transfer as compared with the day 5 transfer when they were calculated per oocyte recovery attempt (\( P < 0.01 \)) (Table V).

**Discussion**

This prospective randomized study has shown that equally high pregnancy and implantation rates can be achieved after transfer of two cleaving embryos on day 3 after ICSI and after transfer of two blastocysts on day 5 after ICSI in a selected population of good-prognosis patients (cases in which \( \geq 8 \) two-pronucleated zygotes are available on the day following ICSI). The implantation rate of day 3 fresh embryos (35%) was higher as compared with previous studies in which embryos transferred on day 3 were evaluated only at the cleavage stages (Scholtes and Zeilmaker, 1996; Marek et al., 1999; Coskun et al., 2000; Hsieh et al., 2000; Huisman et al., 2000; Milki et al., 2000; Langley et al., 2001) with the exception of one study reporting success rates in an oocyte donation programme (Schoolcraft and Gardner, 2000). Hence, the combination of pronuclear zygote scoring with cleavage-stage evaluation seems to perform as well as blastocyst formation. In fact, the morphology of human pronuclear zygotes has been shown to be positively related to blastocyst development (Scott et al., 2000), whereas day 3 morphology alone is a poor predictor of blastocyst development in extended culture (Graham et al., 2000). However, the pronuclear score was in agreement with the day 3 embryo score in 84% of the embryos evaluated in this study. Similarly, another recent study showed that the
pronuclear score is a strong predictor of developmental speed and morphology of human cleaving embryos (Tesarik et al., 2002).

It is also known that the implantation rate after blastocyst transfer is influenced by blastocyst quality (Balaban et al., 2000; Gardner et al., 2000b). On the other hand, it appears to be useless to wait up to day 6 if blastocyst quality is not ideal on day 5 (Shapiro et al., 2001). In this study two blastocysts showing the best morphology from the available embryo cohort were always transferred on day 5. No attempt was made to improve blastocyst quality by extending the culture period up to day 6.

The success rates for cryopreserved embryo transfer were also similar for day 3 and day 5 embryos in this study. However, this comparison was flawed by asymmetry of the two groups with only a few cases in which cryopreserved blastocysts were available for transfer. This asymmetry was also responsible for the slightly higher cumulative implantation and birth rates, calculated per embryo transfer, for day 5 embryos as compared with day 3 embryos. These data do not demonstrate any real advantage for day 5 embryos; they simply reflect a ‘dilution’ of higher-chance fresh embryo transfers, highly prevailing in the day 5 group, by the much higher contribution of the lower-chance cryopreserved embryo transfers in the day 3 group.

Yet it was just because of the contribution of the cryopreserved embryo transfers that the cumulative success rates per oocyte recovery attempt turned clearly in favour of the day 3 transfer policy. With equivalent success rates for both the fresh and cryopreserved embryo transfers, the higher number of cryopreserved embryo transfers in the day 3 group led to a significant improvement of per-oocyte-recovery pregnancy, clinical pregnancy and delivery rates as compared with the day 5 group. Because the clinical and laboratory procedures related to embryo formation are more expensive and represent a higher degree of clinical risk and physical discomfort to the patient than the procedures related to embryo storage and transfer, the day 3 transfer policy, in our hands, was clinically more efficient and more cost-effective as compared with day 5 transfers in the selected population of patients analysed.

High implantation rates for day 3 embryo transfer, similar to those observed in this study, have also been reported by other authors using similar embryo selection criteria (Gerris and Van Royen, 2000; Scott et al., 2000; Gerris et al., 2001).

In the current trend towards single embryo transfer, the extended culture and blastocyst transfer are thus not necessary prerequisites any more.

References

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