Early compaction on day 3 may be associated with increased implantation potential

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Objective: To determine whether day 3 embryos exhibiting early compaction have an improved implantation potential compared to embryos without compaction.
Design: A retrospective cohort study.
Setting: Hospital-based academic medical center.
Patient(s): Women <38 years of age undergoing IVF cycles between November 2001 and December 2004 having a day 3 transfer of one or two embryos with >8 cells.
Intervention(s): Standard IVF protocol.
Main Outcome Measure(s): Compaction grading and implantation rates of 1,047 embryos as related to fragmentation of ≥ 8-cell embryos in patients with either 0% or 100% implantation.
Result(s): Compaction grading was strongly associated with implantation potential; however, the direction of this effect depended on the degree of fragmentation. In embryos with <10% fragmentation, implantation rates increased with the degree of compaction (grade 1, 25%; grade 2, 33%; and grade 3, 47%); in embryos with ≥10% fragmentation, the effect was reversed (grade 1, 38%; grade 2, 20%; and grade 3, 9%).
Conclusion(s): Assessing the degree of compaction can be a valuable addition to traditional morphologic assessment in identifying optimal embryos for transfer on day 3. (Fertil Steril® 2006;86:1386–91. ©2006 by American Society for Reproductive Medicine.)

Key Words: Embryo compaction, embryo fragmentation, IVF, implantation rates

Attempts to maximize IVF pregnancy rates (PR) were initially achieved by transferring greater numbers of embryos. This approach had the negative side effect of increasing the incidence of high-order multiple gestations (1–5). To maintain PRs, and still reduce high-order multiple gestations, there has been a progressive move toward decreasing the number of day 3 embryos transferred (6), as well as performing day 5 transfers involving only one or two blastocysts (7, 8). However, blastocyst transfer may not be ideal in all cases, and may compromise a successful outcome that would otherwise be achieved after a day 3 transfer (8, 9). Therefore, continuing to optimize selection of the most developmentally competent embryos for day 3 transfer remains an important goal in improving IVF implantation rates.

Routine morphologic assessment on day 3 includes cell number (10), extent of fragmentation (11), and degree of asymmetry (12), all of which have been shown to be correlated with PRs after day 3 transfer. Although these correlations are well established (12), many embryos that appear viable on day 3 by traditional assessment fail to implant (13). To supplement traditional means of assessment, several studies have investigated embryonic developmental milestones as an additional means to determine developmental competence.

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Embryos that undergo early cleavage have been associated with higher implantation rate and PR (3, 14, 15). Nucleolar alignment (16) and pronuclear morphology (16–19) have also been postulated as important developmental markers. In addition, combined methods of scoring with differential weighting of these morphologic features have been proposed (20, 21) and correlated with improved implantation rate and PR (20) when compared with traditional assessment (21, 22).

Another potential marker of embryonic developmental competence is the early formation of tight junctions as the process of compaction gets underway. Compaction typically occurs on day 4 as the embryo proceeds to the morula stage (23). Because the degree of compaction on day 4 has been correlated with implantation potential (24), the possibility exists that early compaction on day 3 may also represent a predictor of enhanced developmental competency. Therefore, the purpose of the present study was to determine whether or not day 3 embryos that exhibit early compaction have an improved implantation potential compared to those embryos with little or no compaction.

MATERIALS AND METHODS

Study Entry Criteria

Entry criteria for the cycles included in this study were as follows: IVF cycles between November 2001 and December 2004, using G1.2 or G1.3 media for embryo culture from days 1–3, maternal age <38 years, and day 3 transfer of one

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or two embryos having ≥8 cells on day 3. To assess definitively the fate of each transferred embryo, only cycles resulting in either 0% or 100% implantation were included. Implantation was defined as the presence of a viable fetal heart at 12 weeks of gestation. Therefore, all embryos could be included in the analysis, even in cases where the transferred embryos exhibited different compaction grades.

After appropriate institutional review board approval for chart review was obtained, a total of 611 cycles were identified as meeting these inclusion criteria.

Stimulation Protocols

Patients with normal clomiphene citrate (CC) challenge testing (generally FSH levels <10 mIU/mL) underwent controlled ovarian stimulation with luteal down-regulation using leuprolide acetate (LA; Lupron; TAP Pharmaceuticals, Lake Forest, IL). Leuprolide acetate was begun either a week after documentation of urinary LH surge or the day after a midluteal P determination, and was continued until at least day 2 of menses. Baseline ultrasonography and blood testing were then performed to document that no cysts >3 cm were present, E_2 was <50 pg/mL, and P was <1.5 ng/mL. Alternatively, in patients with histories of poorer gonadotropin responses or FSH levels ≥10 mIU/mL, "poor responder" protocols were used. The most usual protocol used was either a microdose lupron protocol with lupron 0.05 mg SC twice a day started cycle day 1 of a period after oral contraceptive (OC) pill lead in and baseline ultrasound testing performed on day 2 or, alternatively, a GnRH antagonist (GnRH-a) protocol using an OC pill for 3 weeks, then baseline ultrasound testing cycle day 2, with GnRH-a initiation at a dose of 0.25 mg/day SC starting stimulation day 6.

When baseline criteria were met, gonadotropin therapy (either Gonal-F, Serono Laboratories, Inc., Rockland, MA or Follistim, Organon, Roseland, NJ) with or without hMG (Humegon: Organon, Pergonal, or Repronex: Ferring Pharmaceuticals Inc., Suffern, NY) was begun. Stimulation was generally achieved using single or divided daily dosing of between 2 and 8 ampules/day, as appropriate, depending on patient age and anticipated response. Monitoring of follicle growth was achieved using ultrasound, and serum E_2 levels were measured starting on stimulation day 6 and then every 1–3 days as indicated. A dose of 10,000 IU of hCG (Profasi: Serono) was administered IM when two follicles reached a maximum diameter of >20 mm (mean 16.5 mm) and the E_2 concentration was >500 pg/mL. Transvaginal oocyte retrieval was performed 36 hours after hCG administration in the standard fashion with IV general anesthesia or, in some cases, spinal anesthesia as indicated.

Oocyte Fertilization, Embryo Culture, Transfer, and Outcome Assessment

Within 4–6 hours of retrieval, oocytes were inseminated in groups of 3–5 in 1 mL Ham's F10 medium supplemented with 5% human serum albumin (InvitroCare Inc., Frederick, MD) or were injected with a single sperm. After identification of two pronuclei (PN) at the fertilization check 16–18 hours after insemination or intracytoplasmic sperm injection (ICSI) on day 1, zygotes with 2 PNs were cultured individually in 25 μL of growth medium (G1.2 or G1.3; Scandinavian IVF Science/Vitrolife, Gothenburg, Sweden) overlaid with 8 mL of oil in Falcon 1007 culture dishes (Becton Dickinson Labware, Franklin Lakes, NJ).

All cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2 in air. On day 3, the morphology of each embryo was assessed using standard criteria (12) 68–72 hours after insemination (70.7 ± 1.5 hours after insemination, mean ± SD). Fragmentation was graded as <10%, 10%–25%, and >25% of the blastomere volume; blastomere asymmetry was graded according to uniformity in size and shape of the blastomeres as exhibiting no asymmetry, moderate asymmetry, and severe asymmetry.

Embryos having the optimal cell number, the lowest percentage of fragmentation, and the lowest asymmetry in a given cohort were selected for transfer. Other characteristics being equal, preference was given to embryos having eight cells than to those having more than eight cells. The number of embryos transferred to a given patient was determined by the number and quality of embryos she had available, the patient's age, and her prior clinical history. Transferred embryos were photographed within the 2 hours before transfer and photographs were stored in the medical record.

Luteal P supplementation was initiated the day after oocyte retrieval and was achieved using one of three regimens: [1] daily IM P (50 mg); [2] daily vaginal gel (8% P [Crinone; Wyeth-Ayerst, Madison, NJ]); or [3] twice daily P suppositories (50–100 mg). Embryo transfer was performed with a Wallace catheter (Marlow/Cooper Surgical, Shelton, CT). For difficult transfers, a Marrs no. 4 or Marrs no. 5 embryo transfer catheter (Cook Ob/Gyn, Spencer, IN) was occasionally used.

Clinical pregnancies were identified by the presence of a gestational sac on ultrasonography 5 weeks after oocyte retrieval. The implantation rate was calculated as the number of fetal hearts present at 12 weeks of gestation, divided by the number of embryos transferred, multiplied by 100.

Compaction Grading

Embryo images (n = 1,047) in archived photographs were evaluated retrospectively for degree of compaction by an experienced embryologist who was blinded to pregnancy outcomes. Compaction was graded into one of three categories as follows: grade 1 (no compaction: blastomeres spherical with no evidence of membrane fusion); grade 2 (some compaction: some membrane fusion evident but the number of blastomeres easily countable); and grade 3 (full compaction: extreme membrane fusion making it very difficult to count the number of blastomeres present). Embryos were