Incidence and development of zygotes exhibiting abnormal pronuclear disposition after identification of two pronuclei at the fertilization check

David E. Reichman, M.D., Katharine V. Jackson, B.A., and Catherine Racowsky, Ph.D.

Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital, Boston, Massachusetts

Objective: To determine the incidence, developmental potential, and clinical implications of embryos having one pronucleus (1PN) or three pronuclei (3PN) at early cleavage, despite exhibiting 2PN at the fertilization check.
Design: Retrospective cohort study.
Setting: Hospital-based academic medical center.
Patient(s): All IVF cycles from January 2006 through May 2008 having 2PN zygotes that subsequently transitioned to 1PN or 3PN before cleavage, matched to cycles having 2PN zygotes progressing to cleavage without intervening abnormal pronuclear disposition.
Intervention(s): Standard IVF protocol.
Main Outcome Measure(s): Incidence, day 3 development, and implantation rates of 2PN zygotes transitioning to 1PN and 3PN states before cleavage, compared with normal embryos.
Result(s): The incidences of 1PN and 3PN zygotes were 2.9% and 0.4%, respectively. Both types of abnormal zygote showed slower day 3 cleavage, although only the 1PNs exhibited higher fragmentation and asymmetry compared with controls. The 1PN zygotes had a 6.4% implantation rate and viable pregnancy rate of 1.3%. Of the nine 3PN zygotes transferred, none implanted.
Conclusion(s): Two-pronuclear zygotes transitioning through 1PN or 3PN states tend to develop into poorer-quality embryos than 2PN control zygotes. Patients should be counseled regarding the very low likelihood of viable pregnancy after transfer of these abnormally developing zygotes. (Fertil Steril® 2010;94:965–70. ©2010 by American Society for Reproductive Medicine.)

Key Words: Unipronuclear, 1PN, tripronuclear, 3PN, early cleavage, IVF, ICSI, zygote

Several studies have investigated the incidence, origin, development, and chromosomal composition of abnormal pronucleate zygotes (1–3). Single-pronucleate (1PN) zygotes are thought to be secondary to either parthenogenetic activation of the oocyte or abnormal syngamy of pronuclei, whereas tripronucleate (3PN) zygotes result from either dispermy or failure of extrusion of the second polar body. Although such embryos are usually discarded in IVF programs, healthy children have been born after transfer of 1PN embryos (4). It has become the practice of several centers to transfer such embryos in the absence of suitable alternatives.

Studies, however, have focused on zygotes which, at ~16–18 hours after insemination, exhibited abnormal pronuclear number without ever being identified as having a normal two-pronucleate (2PN) state. In our experience, we have occasionally noted zygotes which exhibited evidence of normal fertilization with two pronuclei and later went on to display one pronucleus or three pronuclei when checking for early cleavage (~24–27 hours after insemination). We hypothesized that such zygotes should be classified as "abnormal" and would have poorer developmental potential and implantation rates than zygotes with two pronuclei. Upon our review of the existing literature, we could find only one study mentioning this phenomenon in a single fertilized oocyte (5) and no studies that examined the incidence, developmental fate, or suitability for transfer of these zygotes.

Therefore, the purpose of the present study was to discover the incidence of these abnormally developing zygotes in our standard IVF population, to track their development based on cell number, fragmentation, and asymmetry on day 3, and to determine their suitability for transfer.

MATERIALS AND METHODS

This study was approved by the Partners' Healthcare Institutional Review Board for chart review.

Cycle Inclusion Criteria

The index cycles identified for this study were culled after review of all IVF cycles (either standard insemination or intracytoplasmic sperm injection [ICSI]) performed in our program from January 2006 through May 2008 in which the embryos were evaluated for early cleavage (23.7 to 26.9 hours after insemination, mean time 25.1 hours) and for which development was followed in culture to day 3 (n = 2,859 cycles).

Received February 16, 2009; revised and accepted April 8, 2009; published online May 23, 2009.
D.E.R. has nothing to disclose. K.V.J. has nothing to disclose. C.R. has nothing to disclose.
Reprint requests: Catherine Racowsky, Ph.D., Brigham & Women's Hospital, 75 Francis Street, ASB 1+3, Rm 08, Boston, MA 02115 (FAX: 617-975-0825; E-mail: cracowsky@partners.org).

Clinical Protocols

The patients typically had normal clomiphene citrate challenge testing (generally FSH levels <10 mIU/mL) and underwent controlled ovarian stimulation with luteal down-regulation using leuprolide acetate (Lupron; TAP Pharmaceuticals, Lake Forest, IL). Leuprolide acetate was begun either 1 week after urinary LH surge or the day after midluteal P determination, and was continued until at least day 2 of menses. Baseline ultrasonography and blood testing were performed to document no ovarian cysts >3 cm, E_2 <50 pg/mL, and P <1.5 ng/mL. Alternatively, in patients with histories of poor gonadotropin response or high levels of FSH (≥10 mIU/mL), "poor responder" protocols were implemented. Although these protocols varied, such protocols were typically either microdose lupron with 0.05 mg SC leuprolide acetate twice daily starting cycle day 1 after an oral contraceptive (OC) lead-in with baseline ultrasound testing performed on day 2, or a GnRH antagonist (GnRH-a) protocol using OC pills for 3 weeks with baseline ultrasound testing cycle day 2 and GnRH-a starting at a dose of 0.25 mg/day beginning stimulation day 6.

When baseline criteria were met, gonadotropin therapy (either Gonal-F [Serono Laboratories, Rockland, MA] or Follistim [Organon, Roseland, NJ]) with or without hMG (Humegon [Organon], Pergonal, or Repronex [Ferring Pharmaceuticals, Suffern, NY]) was begun. Stimulation was accomplished using either a single dose or divided daily doses of between 2 and 8 ampules/day, depending on patient age and anticipated response. Monitoring of follicles was achieved using ultrasound. Serum E_2 levels were measured, in general, on stimulation day 6 and then every 1–3 days as indicated. A 10,000 IU dose of hCG (Profasi; Serono) was administered IM when the two lead follicles had a mean diameter of >16.5 mm and the E_2 concentration was >500 pg/mL. Retrieval was performed transvaginally 36 hours after hCG administration in the standard fashion using either IV or regional anesthesia.

One day after oocyte retrieval, luteal P supplementation was begun using one of three regimens: 1) daily IM P (50 mg); 2) daily 8% P vaginal gel (Crinone; Wyeth-Ayerst, Madison, NJ); or 3) twice daily vaginal 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.

Laboratory Protocols

All gametes and embryos were maintained at 37°C in a humidified atmosphere of 5% CO_2 in air. Within 4–6 hours of retrieval, oocytes were inseminated in groups of 3–5 in 1 mL Ham F10 medium supplemented with 5% human serum albumin (Invitro Care, Frederick, MD) or were fertilized (within 3–5 hours after retrieval) via ICSI. Zygotes having 2PN at the fertilization check 16–18 hours after insemination or ICSI were cultured individually in 25 μL of growth medium (G1.2, G1.3 [Scandinavian IVF Science/Vitrolife, Gothenburg, Sweden], or Life Global [IVF Online, Toronto, Canada]) overlaid with 8 mL oil in Falcon 1007 culture dishes (Becton Dickinson Labware, Franklin Lakes, NJ). Early cleavage check was carried out approximately 24–27 hours after insemination to assist in identifying those embryos with maximal implantation potential (6–8).

On day 3, the morphology of each embryo was assessed using standard criteria, with fragmentation and asymmetry being graded with numerical scores (9). Fragmentation scores of 0 through 4 were awarded to each embryo, where each score correlated to 0, 1%–9%, 10%–25%, 26%–50%, or >50% fragmentation, respectively. Blastomere asymmetry was graded using a numerical score of 1 through 3 according to uniformity in size and shape, where a score of 1 represented perfect symmetry, 2 moderate asymmetry, and 3 severe asymmetry. A team of six to eight embryologists, who participate in proficiency testing to ensure consistency in grading, performed the embryo assessments in our lab.

Embryos with the lowest percentage of fragmentation, lowest asymmetry, and optimal number of cells were selected for transfer. Preference was given to eight-cell embryos when available, other characteristics being equal. Number of embryos transfered was determined according to number and quality of available embryos, patient age, and prior clinical history.

Index Zygotes

All 2PN zygotes at the fertilization check (n = 23,293) were screened to identify those with one pronucleus or three pronuclei at early cleavage.

Control Zygotes

Two types of control groups of zygotes were assessed for each of the 1PN and 3PN datasets to compare embryo quality, implantation rates, and pregnancy rates with each type of index zygote: 1) internal control groups, composed of those 2PN zygotes from the same cycles as either the 1PN or 3PN zygotes but not exhibiting abnormal pronuclear number at early cleavage check; and 2) external matched-pair control groups, composed of zygotes from cycles performed during the same time period but with no 1PN or 3PN zygotes. These external control groups were obtained by matching each cycle having a 1PN or 3PN zygote with a control cycle for patient age, date of embryo retrieval, number of eggs retrieved, and number of embryos transferred. For accurate comparison, only control zygotes exhibiting 2PN or 0PN (rather than two or more cells) at early cleavage were selected for analysis. By creating this matched external control group we hoped to eliminate a source of bias, given the possibility that patients who produce 1PN or 3PN zygotes might not be representative of a normal IVF population.

Outcome Variables Assessed

Index zygotes were compared with zygotes in the internal and external control groups for distributions according to cell