Value of transferring embryos that show no evidence of fertilization at the time of fertilization assessment

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Objective: To determine the value of transferring embryos formed from nonpronuclear (0PN) zygotes.
Design: A case-control study.
Setting: Not applicable.
Patient(s): The current study was a retrospective analysis of embryo transfers of just 0PN embryos using fresh cleavage-stage embryos (0PN cleavage fresh), frozen-thawed cleavage-stage 0PN embryos (0PN cleavage frozen), and frozen 0PN blastocyst-stage embryos (0PN blast frozen).
Intervention(s): To study the effect of 0PN transfer, comparison groups were used: fresh cycles of 2PN (2PN cleavage fresh-C) and frozen-thawed cycles cleavage-stage (2PN cleavage frozen-C) and blastocyst-stage (2PN blast frozen-C). Comparison groups were matched for cycle and patient characteristics to the 0PN group.
Main Outcome Measure(s): Implantation rate (IR), pregnancy rate, and transferable embryo rate.
Result(s): For fresh cycles, the IR in the 0PN cleavage fresh was lower than that in the 2PN cleavage fresh-C (8.04% vs. 19.50%, respectively). For frozen-thawed cycles, the IR in the 0PN cleavage frozen was lower than that in the 2PN cleavage frozen-C (15.38% vs. 28.24%, respectively), but the IR in 0PN blast frozen was comparable to that of 2PN blast frozen-C (39.56% vs. 48.18%, respectively).
Conclusion(s): Transfer of 0PN embryos from fresh or frozen-thawed cycles results in pregnancies and live births. Nonpronuclear embryos have a lower IR than 2PN embryos, but if the embryos are cultured to the blastocyst stage and then are frozen, their IRs approach that of 2PN embryos in subsequent frozen-thawed cycles. The culture of 0PN embryos to the blastocyst stage may select for embryos with a near-normal IR. (Fertil Steril® 2015;104:607–11. ©2015 by American Society for Reproductive Medicine.)
Key Words: 0PN, embryo transfer, implantation rate, blastocyst culture

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During in vitro fertilization-embryo transfer (IVF-ET) treatment for infertility, assessment of fertilization is generally performed 16–19 hours after insemination, with the presence of two pronuclei (2PN) indicating normal fertilization. Approximately 20%–30% of mature oocytes do not show evidence of fertilization (no pronuclei visible; 0PN) under light microscopy (1). Some oocytes without evidence of fertilization (0PN embryos) show cleavage by the next day's observation and continue to cleave in subsequent culture; some of them form embryos with a similar morphology to embryos with 2PN. Embryos that show normal fertilization, with 2PN and two polar bodies, are preferred for transfer in IVF-ET cycles. In practice, however, there are sometimes insufficient 2PN embryos for transfer. Under such circumstances, it is uncertain whether 0PN embryos with normal morphology should be used for transfer. Studies involving 0PN ET are rare. Manor et al. reported that a total of six 0PN embryos were transferred into three patients during fresh cycles, resulting in one ongoing pregnancy, although the pregnancy

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outcomes were not reported (2). Burney et al. reported that a healthy infant originating from a 0PN embryo was delivered; no additional live births from 0PN ETs have been reported (3). Several experimental studies have been conducted on the chromosomes of 0PN embryos (2, 4–6). Manor et al. showed that 57% (13/23) of 0PN embryos were normal diploid embryos (2), while two other studies reported 3% (1/30) and 0 (0/4) normal diploid embryos (4, 6); these different results may have been related to the origin of the 0PN embryos from patients with different characteristics. Owing to the limited number of studies focusing on 0PN embryos, as well as the low numbers of cases in these studies, some reproductive centers discard 0PN directly after examining the ova for signs of fertilization. Other reproductive centers continue to culture 0PN embryos and observe their potential for development, allowing transfer when necessary (2, 3). Is there value in transferring 0PN embryos? What about the risks of 0PN ET? To address these issues, this study retrospectively analyzed the clinical outcomes of transfer of 0PN embryos in fresh cycles and frozen-thawed cycles among patients undergoing IVF-ET treatment (including conventional IVF and conventional intracytoplasmic sperm injection [ICSI]) in the Third Hospital, attached to Beijing University, between January 2009 and December 2013. This is one of the first reports with sufficient cases and a detailed analysis of the results of 0PN transfer and provides a guide for clinical practice.

MATERIALS AND METHODS

Patients and Cycles

This was a retrospective analysis of the clinical outcomes of embryos that failed to show evidence of fertilization at fertilization check but that later cleaved and developed into embryos (0PN embryos). A total of 48,307 fresh ETs and 20,875 frozen-thawed ETs in patients undergoing IVF-ET treatments (including conventional IVF and ICSI) were included from this infertility center. The fresh cycles comprised 159 transfers of 0PN cleavage-stage embryos (0PN cleavage fresh). Among the frozen-thawed cycles, cleavage-stage ETs of 0PN embryos (0PN cleavage frozen) occurred in 39 cycles and 0PN blastocyst-stage embryos (0PN blast frozen) were transferred in 82 cycles. In vitro maturation cycles, rescue ICSI cycles (after failure of IVF fertilization), artificial oocyte activation cycles, and preimplantation genetic diagnosis cases were excluded.

The participants underwent controlled ovarian hyperstimulation with a GnRH agonist or GnRH antagonist protocol, as described elsewhere (7). Ovarian follicle development was monitored on the basis of serum E_2 levels and transvaginal ultrasound measurements. When at least one follicle reached a mean diameter of 18 mm and the E_2 concentration was >500 pg/mL, 10,000 units of urinary hCG (Serono) were administered before ultrasound-guided oocyte retrieval. Luteal support was started on the day after oocyte retrieval using 60 mg of P.

The study was approved by the Ethics Committee of Beijing University Third Hospital (reference no. 20080613), and all patients signed written informed consent.

Laboratory Protocols

Between 36 and 38 hours after hCG administration, the oocytes were retrieved and fertilized using conventional IVF or ICSI. In conventional IVF cycles, oocytes were inseminated 3–4 hours after oocyte retrieval. Spermatozoa were collected using the swim-up technique with 50,000 motile sperm cells/mL in the insemination dish. During ICSI cycles, the removal of cumulus cells from oocytes was performed 2 hours after retrieval, and ICSI was performed as described elsewhere (8). Normal fertilization was assessed by the presence of 2PN 17–19 hours after insemination. Embryos were grouped into 2PN, 1PN, >2PN, and 0PN. Available embryos were transferred 72 hours after oocyte retrieval. Serum hCG concentration was measured 2 weeks after ET, and clinical pregnancy was defined as the presence of a gestational sac on ultrasound examination on day 35 after transfer. Supernumerary transferable embryos (including 0PN embryos) were cryopreserved on day 3 or days 5–6 after the fresh ET. Slow-freezing was performed if more than five useful supernumerary cleavage-stage embryos existed after fresh ET. If there were less than six embryos cultured to the blastocyst stage, blastocyst vitrification was done if a blastocyst formed. The slow-freezing and vitrification protocols were performed by standard procedures, as described elsewhere (9, 10). For evaluation of the ability of cleavage-stage 0PN embryos to grow into a blastocyst, the study compared blastulation and transferable blastocyst rates between supernumerary cleavage-stage 0PN and 2PN embryos.

The definitions used in the current study were as follows: day 3 embryos with more than four cells and $\leq$30% fragmentation were defined as transferable embryos. The 2PN rate was calculated as the number of 2PN zygotes divided by the number of inseminated oocytes (inseminated oocytes = oocytes retrieved in IVF + metaphase II in ICSI). The 2PN cleavage rate was calculated as the number of 2PN cleavage zygotes divided by the number of 2PN zygotes. The 2PN transferable embryo rate was calculated as the number of transferable 2PN embryos divided by the number of 2PN zygotes. The same definitions were used for 0PN embryos. Embryos that developed to the blastocyst stage were scored according to the criteria used by Gardner et al. (11). Blastocysts that reached grade 3BB or better quality were defined as transferable blastocysts. Clinical pregnancy rate (PR) was defined by the detection of a gestational sac on ultrasound examination on day 35 after transfer. The implantation rate (IR) was calculated as the number of gestational sacs divided by the number of embryos transferred.

Cycle Matches

To study the effect of 0PN transfer, comparison groups were used from fresh cycles (159 cleavage-stage, 2PN cleavage fresh-C) and frozen-thawed cycles (39 cycles cleavage-stage, 2PN cleavage frozen-C; and 82 cycles blastocyst-stage, 2PN blast frozen-C, respectively). Comparison groups were established to study whether or not the outcomes of 0PN cleavage-stage ET were related to the origin of embryos from 0PN zygotes. Therefore, the patient characteristics were aligned between the 0PN groups and comparison groups. The