Early pronuclear breakdown is a good indicator of embryo quality and viability

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Objective: To examine whether the timing of pronuclear breakdown can be a predictor of embryo quality and viability.
Design: Retrospective comparison of the development and quality of early and late developing zygotes.
Setting: Infertility and endocrinology unit in a university hospital.
Patient(s): One thousand seven hundred eighty-two zygotes obtained in 383 consecutive IVF cycles.
Intervention(s): Culture of all fertilized embryos.
Main Outcome Measure(s): Number of fertilized zygotes showing early pronuclear breakdown at 22–25 hours postinsemination, embryo quality, pregnancy rates (PR), implantation rates.
Result(s): Early pronuclear breakdown embryos had a significantly higher cell number (4.4 ± 1.2) compared with the late pronuclear breakdown embryos (3.6 ± 1.4). When comparing the frequency of the early pronuclear breakdown embryos according to the method of fertilization, we failed to find any significant difference between the IVF (37.1%) and the intracytoplasmic sperm injection (ICSI) (41.1%) groups. The transfer of early pronuclear breakdown embryos resulted in a significantly higher clinical pregnancy rate than those with late pronuclear breakdown (48.3% vs. 27.3%). The implantation rate was higher in the early pronuclear breakdown group than in the late pronuclear breakdown group (26.5% vs. 15.1%).
Conclusion(s): Early pronuclear breakdown is a strong indicator of embryo viability, and may be used as an additional criterion in the selection of embryos for transfer. (Fertil Steril® 2005;84:881–7. ©2005 by American Society for Reproductive Medicine.)

Key Words: Early pronuclear breakdown, early cleavage, embryo quality, pregnancy rates, implantation rate

In vitro fertilization treatment has become a widely used procedure during the past 20 years in most countries. As a result of the more effective ovarian stimulation protocols and of improved fertilization and embryo culture techniques, patients may produce more good-quality embryos. Nevertheless, the implantation rate of individual embryos still remains low. To improve the efficacy of IVF treatments, most IVF units transfer more than one embryo at a time, which results in higher rates of multiple pregnancies. To increase the pregnancy rates (PR) and reduce the occurrence of multiple pregnancies, there is a need to transfer less but more viable embryos.

Several embryo transfer strategies have been developed, which have led to a widening of the range of morphological features that refer to embryo viability. The timing of the first cell cycle could be an important characteristic of the preimplantation human embryo. The duration of the full cell cycle seems to be approximately 22–24 hours. However, considerable differences from this average have been observed (for review, see Plachot) (1).

The G_1 phase of the first cell cycle begins approximately 3 hours after insemination with completion of the second meiotic division and the extrusion of the second polar body. This phase last about 6 hours and is followed by the S phase, in which the chromosomes replicate. DNA synthesis begins between 8 and 14 hours, terminates between 14 and 24 hours postinsemination, and is followed by the G_2 phase, which last 5–6 hours (2).

The M phase of the first cell cycle starts with the breakdown of the pronuclear membrane and terminates with the completion of the first cleavage. The duration of the M phase is 3–4 hours, and it is relatively constant (3, 4). The earliest pronuclear breakdown was observed 18 hours postinsemination, and the latest time was between 30 and 31 hours postinsemination (3–6).

Similarly, the time of the first cleavage can be between 20 and 33 hours postinsemination (4, 5). The zygote-to-zygote variation in the time of pronuclear breakdown or the first cleavage can be more than 12 hours.

Shoukir and colleagues (7) were the first to demonstrate that human embryos that had undergone the first cleavage cycle by 25 hours postinsemination had a better viability than those zygotes that had the first cleavage later.

Since that time, several investigators have reported better PRs and implantation rates when transferring early cleaving embryos (8–12). It seems that early embryo cleavage is a good indicator of embryo viability and may

Received November 8, 2004; revised and accepted March 3, 2005.
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be used as a selection factor both in conventional IVF (7) and intracytoplasmic sperm injection (ICSI) treatments (8).

The period within which assessment of the early cleavage is recommended varies between 24 and 29 hours postinsemination (6, 7, 10, 12). Lundin and colleagues (13) found a higher rate of early cleaving embryos in ICSI treatment than in IVF treatment and raised the question as to whether IVF embryos should be assessed for early cleavage later than ICSI embryos. However, this observation has not been confirmed by other researchers (10, 14).

Early cleaving embryos had higher numbers of blastomeres and better morphology on day 2 than embryos without early cleavage (13, 15, 16). Early cleaving has also been reported to result in a higher blastocyst formation rate (6, 17, 18).

The most spectacular demonstration of the higher implantation potential of the early dividing embryos was made in elective single embryo transfer programs (15, 18). In these studies the clinical PR in the early cleaving group was almost double that in the late cleaving group.

These studies clearly demonstrate that early cleavage is a strong indicator of embryo viability and implantation potential and that it can be an important embryo selection factor for IVF or ICSI embryos. On the other hand, it has been demonstrated in previous studies that the interval between pronuclear breakdown and first cleavage are relatively constant (~3 hours) (3, 4). Only two recent studies took into consideration the occurrence of pronuclear breakdown when assessing the zygotes for early cleavage. They found a significant positive relationship between early two-cell development and pronuclear breakdown with subsequent embryo development, embryo quality, and blastocyst formation (6, 14). However, most assessments of early embryonic development were performed between 25 and 28 hours postinsemination, which is an optimal interval for early cleavage assessment but may be too late for observing early pronuclear breakdown.

On the basis of these observations, we introduced the assessment of early embryonic development between 22 and 25 hours postinsemination into our routine IVF practice. The aims of this retrospective analysis were [1] to investigate whether early pronuclear breakdown could predict good embryonic development of individual embryos on days 2 and 3 postinsemination; [2] to compare the effect of the method of fertilization (IVF or ICSI) on the timing of pronuclear membrane breakdown; and [3] to investigate the effect of transferring early pronuclear breakdown embryos on the main outcomes of IVF/ICSI cycles.

MATERIALS AND METHODS

Source of Embryos

This study was performed with the patients entering the IVF–embryo transfer program of the Division of Assisted Reproduction, First Department of Obstetrics and Gynaecology, Semmelweis University School of Medicine, Budapest, Hungary, between October 2001 and February 2004. The IVF treatments without normal (2 pronuclear) fertilization and those performed for preimplantation genetic diagnosis were excluded from this study. In total, 1,782 fertilized oocytes from 383 IVF/ICSI cycles with embryo transfer were included in this retrospective analysis.

Ovarian Stimulation

Gonadotropin-releasing hormone-agonist "long protocol" or multiple dose/single dose GnRH-antagonist regimen was used for ovarian stimulation. Using the long protocol, pituitary down-regulation was achieved with the GnRH-agonist triptorelin (Decapeptyl; Ferring, Kiel, Germany), at a dose of 0.1 mg/day from the midluteal phase of the cycle preceding the treatment cycle. In the multiple dose GnRH-antagonist "Lübeck protocol" (19), 0.25 mg/day cetrorelix (Cetrotide; Serono, Rome, Italy) was administrated from the fifth day of ovarian stimulation. In the single dose "French protocol" (20), 3 mg of cetrorelix was injected on the seventh stimulation day.

Human menopausal gonadotropin (Humegon; Organon, Oss, the Netherlands, or Menogon; Ferring or Merional, IBSA, Lugano, Switzerland) or FSH (Fostimon HP, IBSA or Gonal-F, Serono) was used for ovarian stimulation, which was monitored by daily E_2 measurements and transvaginal ultrasound examination.

Ovulation was induced with 5,000–10,000 IU of hCG (Profasi, Serono) when at least one follicle with a diameter of ≥18 mm, and three or more follicles with a diameter of ≥16 mm were seen on ultrasound, and serum E_2 levels reached 2–300 pg/mL per ≥6-mm follicle. Transvaginal ultrasound-guided aspiration of follicles was performed 36 hours after hCG administration. Micronized P at a dose of three times 200 mg/day (Utrogestan; Besins Iscovesco, Paris, France) was given intravaginally for luteal phase support.

Sperm Preparation and Fertilization

Progressive motile sperm for insemination was isolated by "swim up" technique or by a two-layer Puresperm (Nidacon International, Gothenburg, Sweden) density gradient centrifugation, according to the quality of the native semen sample.

Conventional IVF was performed routinely 6 hours after oocyte collection (day 0). Inseminated motile sperm concentration was $0.1–0.5 \times 10^6$/mL according to the patient age and semen quality. The next morning inseminated oocytes were mechanically denuded of their cumulus cells and placed into culture media individually in separate wells of a four-well dish.

The ICSI treatment was performed 3–8 hours after oocyte collection. The indications for ICSI were [1] $<1 \times 10^6$ progressive motile sperm after preparation, [2] ≤4 oocytes

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