Prediction of embryo developmental potential and pregnancy based on early stage morphological characteristics

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Objective: To analyze the association between morphological details at different stages of culture with blastocyst development, with an aim to improve selection for transfer.
Design: Retrospective audit of data.
Setting: Tertiary referral center and university hospital.
Patient(s): Two hundred sixty-eight couples underwent 357 treatment cycles.
Intervention(s): Oocyte pickups for IVF or intracytoplasmic sperm injection (ICSI) after ovarian stimulation. Embryos were individually cultured and examined on days 0–2 for morphological details and developmental characteristics, and selected for transfer, freezing, or further culture.
Main Outcome Measures: The association of blastocyst development and pregnancy with morphological characteristics.
Result(s): Five morphological characteristics (appearance of the cytoplasm, pronuclei and nucleoli, cytoplasmic deficit, and developmental rate) showed the strongest association with blastocyst development. By combining information from all days of culture into a cumulative score, prediction was greatly improved, compared to only using day 2 morphology. Cytoplasmic dysmorphisms of the oocyte, including accumulation of smooth endoplasmic reticulum, were associated with poor developmental performance. Differential weighting of these characteristics was included in a new embryo scoring system, which showed a strong correlation with implantation.
Conclusion(s): Weighting individual morphological characteristics of zygotes and embryos and combining them into a cumulative embryo score can improve selection of embryos for transfer. (Fertil Steril® 2006;86:848–61. ©2006 by American Society for Reproductive Medicine.)

Key Words: Embryo score, blastocyst, oocyte, zygote, morphology

A reduction of the number of embryos transferred after IVF/intracytoplasmic sperm injection (ICSI) is highly desirable in view of the burden associated with multiple gestation (1). The selection of embryos for transfer into the uterus is a critical step in the IVF/ICSI treatment to reduce the risk of multiple gestation and to maximize the probability of pregnancy, especially when only one embryo is transferred. Although some clinics replace embryos into the uterus on day 2 of development, others prefer replacement on day 3 or, even later, at the blastocyst stage.

One advantage of choosing a later stage of development for replacement is that it allows for selection of embryos that have shown competency to reach developmental milestones (e.g., the maternal–embryonic switch of developmental control) (2). A theoretical disadvantage of later stage transfers is that, because of deficiencies in the present culture systems, some embryos that could have survived in vivo may be lost through attrition in vitro. Also, blastocyst culture places more demand on the resources of the laboratory, as the embryos occupy space in the incubators for longer periods of time and require additional handling. Randomized controlled trials involving unselected patients, comparing transfer at the cleavage stage versus at the blastocyst stage, do not suggest that either alternative is superior in terms of births per oocyte pickup (3). Against this background, there seems to be a case for improving the selection of cleavage stage embryos. Some patients may still request blastocyst transfer and a cleavage stage scoring system is also potentially useful to help counsel such patients on day 2 as to what the chances are for their embryos to develop to blastocyst stage in vitro, and hence to choose between day 2–3 (cleavage stage) embryo transfer versus day 5–6 (blastocyst stage) embryo transfer.

Hundreds of articles have been published on embryo morphology, and there are numerous articles indicating the usefulness of multistage scoring systems (see Ebner et al. [4] for review). However, crude embryo scoring systems are still

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common and further information needs to be disseminated to improve practices.

The aim of the present retrospective study was to analyze how a range of morphological characteristics, used in an initial scoring system, are interrelated and related to the developmental potential of embryos, as well as to determine their usefulness for predicting embryonic development when combined into a summary score. Based on these findings a new, differentially weighted scoring system was developed and its relationship to implantation rates was analyzed.

MATERIALS AND METHODS

Patients and Treatments

During the period January to December 2001, 268 couples underwent 357 oocyte pickups for IVF (n = 170) or ICSI (n = 187) treatment. Embryo transfer was usually done 2 days after oocyte pickup (D2). In the majority of the treatment cycles, a hormonal treatment regimen designated OCP-Long protocol was used. In this protocol, the patients started oral contraceptive (OC) pills (Brevinor 21; Pharmacia Australia, Rydalmere, NSW, Australia) 3–5 days after the onset of menses and continued for 21 days. After taking the OCs for 21 days, administration of GnRH agonist (GnRH-a) was started, either using nafarelin (Synarel, Pharmacia Australia) 200 μg intranasal spray twice a day, or leuprolide acetate (LA) injections (Lucrin; Abbot Australasia, Cronulla, NSW, Australia) 1 mg SC daily.

After 14 days of GnRH-a treatment, ovarian down-regulation was confirmed by measurement of serum E_2 level. If the level of E_2 was >120 pmol/L, GnRH-a treatment continued for another week and the E_2 level was assessed again. Estrogen-producing cysts were aspirated. When E_2 was <120 pmol/L, stimulation with recombinant FSH (Gonal-F; Serono Laboratories, Frenchs Forest, NSW, Australia; or Puregon; Organon Laboratories, Lane Cove, NSW, Australia) was initiated, commonly with a starting dose of 150–225 IU injected SC daily. The FSH dose was adjusted according to the ovarian response, and serial E_2 and transvaginal ultrasound scans were performed every 2–3 days from day 6 of FSH stimulation onward.

When one or more follicles had reached a size ≥18 mm, final oocyte maturation was induced with an SC injection of 10,000 IU of hCG (Profasi; Serono Laboratories or Pregnyl; Organon Laboratories). Oocyte pickup under general anesthesia followed 36 hours later. Embryo transfer was commonly performed on D2. Fourteen days after embryo transfer, serum β-hCG was determined as a pregnancy test (considered positive if >20 IU/L) and a transvaginal ultrasound scan of the uterus was done after 6–7 weeks of amenorrhea to determine whether a clinical pregnancy had been established (intrauterine gestational sac visible).

Because this study was a retrospective audit of already existing clinical practices and collected data, it was not deemed necessary to obtain approval from the University of New South Wales ethical committee (the equivalent of an Institutional Review Board).

Culture Conditions and Scoring

The gametes and embryos were handled and cultured in the Quinn's Advantage (QA) range of media and supplements and additives, all from Sage Biopharma (Gytech, Melbourne, Australia). All media were supplemented with 10 mg/mL of human serum albumin. Egg collection, sperm preparation, and ICSI were done in QA medium with HEPES, whereas fertilization in vitro was done in QA fertilization medium in four-well dishes, with up to six eggs per dish. The ICSI oocytes were cultured individually in 10-μL droplets under oil.

From D1 onward, all zygotes/embryos were individually cultured in droplets. On D1, at about 18 hours after insemination or ICSI, zygotes were moved to 10-μL droplets of QA cleavage medium under oil and scored. Scoring on D2 took place at about 42 hours after insemination. On D3, the embryos were transferred to 10-μL droplets of QA blastocyst culture medium under oil. Embryos were cultured at 37°C either in large incubators in 5% CO_2 in air, or in MINC incubators (Cook IVF, Eight Miles Plain, QLD, Australia) in 6% CO_2, 5% O_2, and 89% N_2. All tissue culture plastics (all Becton Dickinson, North Ryde, NSW, Australia) were prewarmed to 37°C, aspirates were kept in tube warmers at all times, work bench surfaces were heated to 38.5°C to maintain the temperature in the culture dishes at 37°C, the room temperature of the laboratory was 25°–26°C, and laboratory lighting was dimmed.

Oocytes for ICSI were denuded using hyaluronidase (Sage Biopharma), 10 IU/mL, in QA medium with HEPES. Using the initial scoring system, they were scored for characteristics shown in Table 1A, some of which are illustrated in Figure 1. Oocyte–cumulus complexes for IVF were not scored, but anything remarkable about their appearance, such as absence of matrix expansion, was noted on the embryology work sheet. Because oocytes obtained for IVF treatment were not denuded, they could not be scored in the same way as oocytes obtained for ICSI, and hence were not assigned a D0 score.

On D1, at 16–18 hours after insemination, zygotes from IVF were denuded of their granulosa cells (GC) using Flexipets (Cook IVF) to facilitate inspection, and moved to cleavage medium. Later in the day, at 25 hours after insemination, the embryos were inspected for occurrence of syngamy. The zygotes were scored for the characteristics described in Table 1B, some of which are illustrated in Figure 1.

The next day, D2, at 42 hours after insemination embryos were, in most cases, selected for transfer and freezing. Scoring criteria for D2/3 are shown in Table 1C, some of which are illustrated in Figure 1. The morphological characteristics for the D2 score were derived from that described by Mohr et al. (5). The corrected D2 score is the D2 score corrected for multinucleated blastomeres (MNBs), therefore the score