Relationship between pre-embryo pronuclear morphology (zygote score) and standard day 2 or 3 embryo morphology with regard to assisted reproductive technique outcomes

John F. Payne, M.D., Douglas J. Raburn, Ph.D., Grace M. Couchman, M.D., Thomas M. Price, M.D., Margaret G. Jamison, Ph.D., and David K. Walmer, M.D., Ph.D.

Department of Obstetrics and Gynecology, Duke University Medical Center, Durham, North Carolina

Objective: To test the hypothesis that pregnancy rates are low if grade Z1 pre-embryos are not available for transfer and to determine if pronuclear morphology is a better predictor of pregnancy than traditional embryo morphology.
Design: Prospective clinical study.
Setting: Academic human reproduction laboratory.
Patient(s): One hundred couples undergoing IVF with conventional insemination or ICSI.
Intervention(s): Embryo quality was assessed using both pre-embryo pronuclear morphology (zygote scoring or Z-scoring) at the time of fertilization evaluation and standard day 2 and day 3 embryo morphology (number of blastomeres and grading based on degree of fragmentation and blastomere size).
Main Outcome Measure(s): We tested two decision models, one based on Z-scores and another on morphology, to determine which grading system better predicted pregnancy outcomes in assisted reproductive technique. Zygote score and embryo morphology were measured for all embryos and the transferred embryo pool. Implantation and pregnancy rates resulting from the embryo transfers of all cycles were calculated.
Result(s): The Z-score distribution of 552 embryos was 27% Z1, 8% Z2, 50% Z3, and 15% Z4. Z1 and Z3 embryos had significantly ($P\sim.03$) higher quality over Z2 and Z4 embryos. Using the Z-score decision model with Z1 embryos having highest priority for transfer, pregnancy rates were similar between Z1 and Z3 embryos. Using embryo morphology as a decision model, pregnancy rates were highest in transfers containing one or two "best"-quality embryos.
Conclusion(s): Z1 and Z3 embryos had similar morphology and pregnancy rates. The decision model based on the Z-score model was not better than standard embryo morphology in predicting pregnancy outcome. (Fertil Steril® 2005;84:900–9. ©2005 by American Society for Reproductive Medicine.)

Key Words: Assisted reproductive techniques, ART, embryo morphology, embryo quality, fertilization, infertility, IVF, ICSI, nucleoli, pregnancy, pre-embryo pronuclear morphology, pronuclear grading, zygote score, Z-score

The efficiency of IVF and embryo transfer in the human is low, with <30% of embryos that are transferred ever achieving their full developmental potential (1). Because the implantation rates have remained relatively low, the practice of replacing multiple embryos (n = 2–6) in order to increase the likelihood of pregnancy, is common practice. This has led to the occurrence of undesirable high-order multiple pregnancies. To overcome this problem, some countries have restricted the number of embryos that can be replaced to two. Although this will reduce the level of multiple pregnancies, it also reduces the pregnancy rate.

Received January 3, 2005; revised and accepted April 23, 2005.
Presented at the 60th Annual Meeting of the American Society for Reproductive Medicine (ASRM), Philadelphia, Pennsylvania, October 16–20, 2004.
Supported in part by an unrestricted grant from Organon Pharmaceuticals.
Reprint requests: David K. Walmer, M.D., Ph.D., Reproductive Endocrinology and Fertility, Duke University Medical Center, Durham, NC 27710 (FAX: 919-681-7904; E-mail: david.walmer@duke.edu).

Initially, most IVF centers replaced embryos on the second day of culture, at the 2–4-cell stage. By allowing development for an additional day, permitting more critical assessment of the embryos after further cleavage divisions, an increased implantation rate was achieved (2). This system has been widely adopted, with a concomitant increase in implantation rates. For both day 2 and day 3 embryo transfers, embryo selection is based on the key morphologic features of cleaving embryos that have been previously related to increased implantation (3–6).

The morphology of early human zygotes at the 1-cell stage has also been used successfully in an IVF program as a means of embryo selection for both day 1 pronuclear transfers (7) and at the 8-cell stage on day 3 (8). The zygote grading system used by Scott and Smith (9) was based on empirical observations correlated with pregnancy and on previously published observations of zygote morphology (10, 11). The basis of the grading system was a combination

0015-0282/05/$30.00
doi:10.1016/j.fertnstert.2005.04.047

of pronuclear size, nucleoli number and distribution, and cytoplasmic appearance.

In addition, changes in cytoplasmic appearance and progression to first cleavage division were considered. This grading system was used prospectively to increase the incidence of implantation when utilizing day 1 embryo transfers and to select embryos for cryopreservation. Pregnancy rates were equivalent when day 1 and day 3 transfers were compared.

Tesarik and Greco (12) reported a modified grading system in which the nucleoli size, number, and distribution, were utilized in a single-observation scoring. Embryos were then replaced on the third day of culture where the embryo morphology was used as the primary selection criterion. In a retrospective analysis of their data, Tesarik and Greco found a strong association between implantation and the quality of nucleoli within each nucleus of the pronuclear embryos from which the resulting transferred cleaving embryos arose.

The advantages of the Tesarik zygote-grading system over the Scott system are the single observation and fewer parameters for consideration. They later simplified their grading system into either normal or abnormal pronuclear stage pattern and reported that the transfer of only one normal (pattern 0) pronuclear stage embryo was sufficient for the optimal chance of pregnancy (13). Their grading system has been used by others, and they have also reported that embryo transfers including at least one pattern 0 resulted in significantly more pregnancies than transfers without any pattern 0 zygotes (14).

With the introduction of extended culture and blastocyst transfer, reported pregnancy and implantation rates have increased (15). This has led to the ability to select one or two blastocysts for transfer without reducing the chances of pregnancy while minimizing the incidence of high-order multiple pregnancies. However, only 40%–50% of all zygotes placed in extended culture are reported to reach the blastocyst stage and, of these, 30%–40% implant, meaning that even extended culture with blastocyst transfer is inefficient. Because there is clearly a relationship between zygote morphology and the ability to implant, screening embryos at the zygote stage and again at the blastocyst stage could increase the implantation rate, enabling single blastocyst transfers. A second level of embryo screening could also help in selecting patients for extended culture.

Balaban et al. (16) and Zollner et al. (17) described that the pronuclear pattern of the zygote is closely related to blastocyst formation and quality. Others have reported that pronuclear morphology also predicts embryo chromosomal constitution (18–20). A later study reported that in patients younger than 38 years, zygotes with similar pronuclei size and with polarized nuclear polar bodies present the best prognosis based on embryo development and the incidence of chromosomal abnormalities, whereas in patients older than 37 this correlation did not exist (21).

Differential mitochondrial distribution in human pronuclear embryos leads to disproportionate inheritance between blastomeres and diminished ATP-generating capacity and may lead to reduced embryo competence (22). Using a graduated embryo score including pronuclear scoring, first cleavage, and day 3 morphology has been reported to predict blastocyst formation and pregnancy rate from cleavage-stage embryos (23). Pronuclear scoring has been reported to be beneficial in selecting which zygotes are allowed to be cultured for embryo transfer in countries with legal limitations (24).

However, not all studies regarding pronuclear scoring have been favorable. Salumets et al. (25) used two different classification systems and only single embryo transfers and found that although cleavage rates were slower with unpolarized nuclear precursor bodies and morphology was better with halo-positive embryos, there were no significant differences in embryo quality or implantation/pregnancy rates between proposed zygote classes.

Scott et al. (26) described the use of a simple single-observation zygote scoring system modified from an earlier version (27) over 20 consecutive months (613 oocyte retrievals) in an IVF program in which extended culture and blastocyst transfers accounted for ~50% of embryo transfers. Cleaving embryos that were selected initially by zygote morphology and secondarily by morphology on day 3 had increased implantation rates and pregnancy rates (31% and 57%, respectively) as compared to those selected by morphology alone (19% and 33%, respectively).

There was a significant difference between zygote-scored and nonscored cycles on day 3 (pregnancy rate: 57% vs. 33%; implantation rate: 31% vs. 19%) and on day 5 (pregnancy rate: 73% vs. 58%; implantation rate: 52% vs. 39%). Scott's data suggested that zygote scoring can maintain pregnancy rates for both day 3 and day 5 transfers, increase implantation rates, and reduce the numbers of embryos required to achieve a pregnancy.

The objective of our study was to evaluate the human embryo pronuclear morphology (zygote or Z-scores) and cleavage-stage embryo morphology (day 2 and/or 3) in women undergoing assisted reproductive techniques (ART) to test three hypotheses. The first hypothesis was that the transfer of Z1 embryos is more likely to achieve pregnancies than other Z-scores. The second hypothesis was that our Z-scoring data would be comparable to prior studies, specifically those of Dr. Scott. The third hypothesis was that the Z-scoring method is better than standard cleavage embryo morphology in choosing the embryos with the best potential for pregnancy. Our long-term goal was to assist in the selection of viable embryos for embryo transfer in an effort to increase clinical pregnancy rates with the future goal of decreasing multiple gestation rates by transferring fewer embryos to achieve a comparable pregnancy rate.