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Embryo fragmentation as a determinant of blastocyst development in vitro and pregnancy outcomes following embryo transfer

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KEY WORDS

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Objective(s): To determine how the type of embryo fragmentation on day 3 affects progression of human embryos to blastocyst and pregnancy rates following embryo transfer.

Study design: Retrospective analysis of all in vitro fertilization cycles in patients ≤ 40 years of age or younger from January 2002 through December 2003, during which time surplus day 3 embryos were transferred to blastocyst medium for extended culture. All embryos (4 cells or more) not suitable for transfer or freezing 72 hours following in vitro fertilization were placed into microdroplets (60 μ L) of blastocyst medium and cultured for an additional 48 hours to assess blastocyst formation. Normal blastocyst development required blastulation, a visible inner-cell mass, trophoctoderm cells covering 60% of the inner zona surface and thinning of the zona. The rate of blastocyst formation was then analyzed (χ^2 and analysis of variance) against the type of fragmentation 72 hours after insemination. Pregnancy outcomes were analyzed with respect to the pattern of fragmentation in cleaving embryos transferred after 3 days of culture.

Results: A total of 1566 embryos were cultured beyond day 3 of development of which 229 (14.6%) reached the blastocyst stage and were frozen. Embryos exhibiting no fragmentation or type I fragmentation had significantly higher blastocyst development rates (27.9% and 19.9%) than embryos with type 2 or 3 fragmentation (13.9 and 8.8, respectively; $P < .001$). No embryos with type 4 or 5 fragmentation progressed to blastocyst. The average type of fragmentation in transferred embryos correlated with pregnancy outcome and embryo age.

Conclusion(s): More pervasive embryo fragmentation was associated with a decreasing rate of blastocyst development with day 3 embryos. To the extent that blastocyst development rates of day 3 embryos is an index of embryo viability, our findings establish that careful classification of the type of embryo fragmentation is important in selection of day 3 embryos for transfer. Recent reports of associations among embryo fragmentation, aneuploidy, apoptosis, and patient age support these conclusions.

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Our laboratory adopts a standard grading system for day 3 embryos, the elements of which include blastomere number, blastomere size and symmetry, membrane definition, cytoplasmic clarity, cell compaction, and fragmentation. This grading system is weighted toward blastomere number (usually representing 50% of the total possible embryo grade), in accord with the published evidence that this is an important determinant of embryo implantation.^{1,2} Using this system, the difference in average grade of all embryos transferred preceding a pregnancy (4.6) has historically remained higher than the respective average value for transfers preceding a failed conception (4.0; $P < .001$), but the difference in these average grades is relatively low, and we remain constantly faced with discordant pregnancy outcomes (ie, “perfect” embryos transferred with no pregnancies and poor-grade embryos yielding high implantation rates).

Anucleate fragmentation in human embryos has been associated with a lower implantation potential.^{3,4} Accumulating data from preimplantation genetic diagnosis has also established a low likelihood of implantation of aneuploid embryos and a very close association between the amount of embryo fragmentation and chromosome anomalies.⁵ The collective findings infer a close association between embryo fragmentation and implantation and have prompted us to review the fragmentation component of our embryo grading with the view to determining whether assessments of fragmentation should represent more or less of the overall embryo grade. Fragmentation is currently scored relative to the amount (0, 0% to 5%, 5% to 50%, more than 50%) and type, based on criteria detailed previously,³ including:

- Type 1, minimal volume of the embryo affected (less than 5%), and fragments are typically associated with only 1 blastomere.
- Type 2, localized fragments predominantly occupying the perivitelline space and representing 5% to 50% of the embryonic volume.
- Type 3, small fragments distributed throughout the embryonic mass and usually representing 5% to 50% of the embryonic volume.
- Type 4, large fragments distributed throughout the embryonic mass and associated with pleiotropic blastomeres. Usually 50% or more of the embryonic volume is affected.
- Type 5, fragments appear necrotic (grainy/dark cytoplasm), with granularity and cytoplasmic contraction of neighboring blastomeres. More than 50% of the embryonic volume is affected.

Although the proportion of embryonic volume containing fragments currently represents up to 17% of the total embryo grade, the type of fragmentation has been documented but not factored into the grading system. This retrospective study therefore examines the associ-

ation between pregnancy outcomes and the type (as opposed to amount) of fragmentation in transferred embryos.

Material and methods

Study design

To project the relative lethality of the types of fragmentation on preimplantation embryonic development, we analyzed relationships between the type of embryo fragmentation on day 3 of embryo culture and the progression to blastocyst of all embryos 4 cells or greater that were not transferred or frozen by day 3 of culture. Unless stipulated otherwise, the data presented in this study were drawn only from women in whom embryos were transferred were aged 40 years or younger and from January 2002 through December 2003, during which time the same blastocyst culture medium (Irvine Scientific, Irvine, Calif) had been in use. No other restriction on patient or embryo parameters was applied. No institutional review board approval was obtained because of the observational nature of this retrospective data analysis.

Embryo transfer data from the same time period were analyzed to establish the likely relative significance of the (average) fragmentation type on pregnancy outcome. In our comparisons against iterations of fragmentation type, the statistical manipulations of the type score (eg, average) have been performed solely to rank groups of observations relative to (average) fragmentation type (ie, the score assigned to the type [0 to 5] is subjective and not continuously variable), so the score is not otherwise amenable to parametric statistical analysis. Initially, therefore, data on scores of fragmentation type were analyzed to verify (determine) that the assigned type (0 to 5) was aligned with embryo viability. That is, that an embryo with no fragmentation had a higher likelihood of implanting than an embryo with type 1 fragmentation, which in turn had a higher likelihood of implanting than an embryo with type 2 fragmentation, and so on. Average values of the fragmentation type were then reviewed in comparison with other established determinants of pregnancy following embryo transfer (embryo age, number of embryos transferred, blastomere number, embryo grade), and compared between groups of patients who conceived and those who did not.

Results

Development of embryos to blastocyst

Table 1 summarizes outcomes of culture of all surplus day 3 embryos (4 cells or more) following 48 hours of culture in blastocyst medium (Irvine Scientific) between

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