The Prognostic Significance of Day 3 Embryo Cleavage Stage on Subsequent Blastocyst Development in a Sequential Culture System

ROBERT BOOSTANFAR,^{1,2} JOHN K. JAIN,¹ CRISTIN C. SLATER,¹ DAVID E. TOURGEMAN,¹ MARY M. FRANCIS,¹ and RICHARD J. PAULSON¹

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Purpose: To determine prognostic significance of blastomere number on Day 3 of culture upon subsequent blastocyst (BL) development.

Methods: A retrospective analysis was conducted in 37 IVF subjects undergoing standard protocols and BL transfer after sequential embryo culture in P1 and BL media.

Results: Of Day 3 embryos containing 7 or more blastomeres, 68.9% (186/270) developed into BL compared to embryos containing 4–6 blastomeres, 38.1% (56/147), $P < 0.0001$. The majority of BL, 68.9% (168/244), were observed on Day 5. Extended Day 6 culture represented 31.1% (76/244) of all BLs.

Conclusions: The observation of 7 or more blastomeres on Day 3 yielded a significantly greater likelihood of BL development. Embryos containing 4–6 blastomeres are still relatively likely to progress to a BL. Extended culture to Day 6 still yields a significant proportion of BL. Cell cleavage stage on Day 3 appears to be a useful prognostic indicator of subsequent BL development.

KEY WORDS: blastocysts; Day 3 embryo; blastomeres; cleavage stage.

INTRODUCTION

Contemporary improvements in sequential embryo culture to the blastocyst (BL) stage have been successful in the clinical enhancement of pregnancy outcome. In vitro culture of embryos to the BL stage is associated with an increased per embryo implantation rate, subsequently resulting in comparable pregnancy rates with the transfer of fewer embryos compared to Day 3 (1,2).

As the practice of IVF and embryo transfer shifts toward BL culture, it is still unclear which embryos are most likely to reach this advanced stage. Currently there are insufficient data with respect to appropriate selection criteria for patients eligible for BL transfer.

It has not been established which morphologic criteria on Day 3 is a good indicator of subsequent BL formation. Rijnders and Jansen assessed the degree of fragmentation on Day 3 and subsequent BL development (3). Forty-seven percent of Day 3 embryos graded as class I (no fragmentation) and class II (<20% fragmentation) developed into BLs. Twenty-one percent of class III (20–50% fragmentation) and class IV (>50% fragmentation) developed into BLs.

The number of eight-cell cleavage stage embryos on Day 3 has been suggested to be a prognostic guide for continuing culture to the BL stage. Racowsky *et al.* reported that in subjects with ≥ 3 eight-cell embryos on the third day after retrieval, transfer of Day 5 BLs resulted in a decrease in multiple gestations but no difference in ongoing pregnancy rates compared to Day 3 transfer (4). However, in the presence of only 1 or 2 eight-cell embryos on the third day after retrieval, there was no significant difference in pregnancy outcomes whether transfer was performed

¹ Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, Women's and Children's Hospital, University of Southern California Keck School of Medicine, Los Angeles, California.

² To whom correspondence should be addressed at Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, Women's and Children's Hospital, University of Southern California Keck School of Medicine, 1240 N. Mission Road, Room 8K9, Los Angeles, California. 90033; e-mail: drboostanfar@hotmail.com.

Table I. Blastocyst Development on Postaspiration Day 5 or 6 as a Function of Blastomere Number Determined on Day 3

Day 3	% Blast on Day 5	% Blast on Day 6	Total % Blast D5 + D6
2 cell	0% (0/5)	0% (0/5)	0% (0/5)
3 cell	7.0% (1/15)	7.0% (1/15)	13.3% (2/15)
4 cell	18.2% (8/44)	27.2% (12/44)	45.5% (20/44)
5 cell	23.5% (8/34)	11.8% (4/34)	35.3% (12/34)
6 cell	14.4% (12/69)	14.4% (12/69)	34.8% (24/69)
7 cell	50.0% (15/30)	23.3% (7/30)	73.3% (22/30)
8 cell	47.8% (90/188)	17.0% (32/188)	64.5% (122/188)
9 cell	66.6% (2/3)	0% (0/3)	66.6% (2/3)
10 cell	63.9% (30/47)	17.0% (8/47)	80.1% (38/47)
≥12 cell	100% (2/2)	—	100% (2/2)

on Day 3 or Day 5. There appears to be some latitude with respect to these Day 3 morphologic criteria as independent markers of BL development. If this is truly the case, then is it also plausible that Day 3 cleavage stage has some latitude with respect to outcome?

The purpose of this analysis was to determine the prognostic significance of the number of blastomeres present on Day 3 of embryo culture upon subsequent development to the BL stage in a sequential culture system.

MATERIALS AND METHODS

A retrospective chart review during calendar year 1999 was conducted. There were 37 subjects who underwent BL transfer cycles. All subjects were under the age of 35. Thirty-two subjects were oocyte donors and 5 subjects underwent conventional IVF. Of the latter group, two cycles utilized intracytoplasmic sperm injection (ICSI).

All subjects underwent standard controlled ovarian hyperstimulation with leuprolide (Lupron[®], TAP Pharmaceuticals, Deerfield, IL) using long protocol down-regulation followed by hMG or FSH stimulation. Follicular development was monitored with serial transvaginal ultrasound and serum estradiol. When at least two follicles had reached a size of >18 mm, hCG 10,000 IU was administered. Thirty-six hours later, subjects underwent ultrasound guided transvaginal follicle aspiration.

The majority of oocytes obtained were inseminated conventionally with 100,000 sperm/50 μ L drop. In two cases ICSI was performed approximately 4 h after retrieval. Gametes and embryos were cultured in 50 μ L of P1 media (Irvine Scientific, Santa Ana, CA) under oil, with 10% synthetic serum substitute (SSS, Irvine Scientific) for the first 72 h. Embryos were then transferred to BL media (Irvine Scientific) for

the remainder of the culture period. Embryos were cultured until Day 5 or Day 6 and were evaluated for transfer or cryopreservation.

The statistical analysis was performed with STAT-CALC. Statistical significance was determined by Fisher's exact test.

RESULTS

A total of 763 oocytes were obtained, of which 431 fertilized (56.5% fertilization rate). The total number of BLs noted on Day 5 and Day 6 of culture was 244. The percentage of BLs per aspirated oocyte was 32.0% (95% CI: 29–35%) and per fertilized oocyte 56.6% (95% CI: 52–61%).

Table I details the association between the number of blastomeres on Day 3 with the subsequent BL development on Days 5 and 6. Of Day 3 embryos that contained 4–6 blastomeres, 38.1% (56/147) developed into BL; compared to Day 3 embryos that contained 7 or more blastomeres, 68.9% (186/270), $P < 0.0001$. Extended culture of embryos to Day 6 resulted in the development of 76 BLs, representing 31.1% (76/244) of the embryos observed.

A total of 78 BLs were transferred in the 37 cycles (2.1 BL transferred per cycle). The pregnancy rate was 70.3% (26/37, 95% CI: 54–84%). Four pregnancies (15.4%) ended in a spontaneous abortion and there was a 59.5% (22/37, 95% CI: 44–75%) ongoing pregnancy rate. Four pregnancies have ended in term deliveries and the remaining pregnancies are ongoing.

DISCUSSION

Controversy still exists to some extent as to which patients undergoing IVF can have successful BL transfer and which parameters may prospectively predict attainment of the BL stage. Janny and Menezo

reported a reduction in both BL formation and expansion rates with increasing patient age (5). Since increasing female age is known to be associated with decreasing oocyte quality as well as decreased embryo implantation rates this is not a surprising finding (6,7). However, this observation has yet to be confirmed by other investigators. Scholtes and Zeilmaker observed that the number of oocytes retrieved seemed to dictate the chance of BL transfer with respect to the apparent effect of female age (8). Shoukir *et al.* did not find a difference in maternal age between those patients with and without BL development (9).

In trying to predict which cycles are most likely to achieve BL stage embryos, various morphological criteria have been examined. Wiemer *et al.* described BL development in a coculture system, where it was observed that 90% of Day 3 embryos (6–8 blastomeres with minimal or no fragmentation) had developed to the BL stage (10). This was compared to 55% of “sub-optimal” embryos (5–8 blastomeres with 30–50% of fragmentation on Day 3).

We observed a substantial rate of BL development (68.9%) in contemporary sequential culture media from Day 3 embryos that contained 7 or more blastomeres. This was found to be statistically higher when compared to Day 3 embryos that contained 4–6 blastomeres. However, embryos that contained 4–6 blastomeres on Day 3 of culture are still associated with a relatively good likelihood of BL development (38.1%). Our results are consistent with recently published data from a Vero cell coculture system in which there was a strong tendency for higher rates of BL development among embryos with greater number of cells 72 h after retrieval (11).

Extended culture of embryos beyond Day 5 still yields a significant proportion of BL (31.1%). It is our practice to transfer 2 BL whenever the first one reaches the fully expanded state. Other expanded BL are cryopreserved on Day 5 and/or Day 6. It is impossible, on the basis of the data presented here, to assess the relative pregnancy potential of Day 5 versus Day 6 BL. However, pregnancies have occurred after transfer of Day 6 BL.

We conclude that cell cleavage stage on Day 3 is a useful prognostic indicator of subsequent BL

development and that it may act as a barometer of successful BL culture to counsel patients and to guide the embryologist and clinician.

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