# The Influence of Prefreeze Growth Rate and Blastomere Number on Cryosurvival and Subsequent Implantation of Human Embryos

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***Purpose:*** *To determine whether the relatively low implantation rate of cryopreserved Day 2 embryos with only 2 blastomeres can be increased as a consequence of increasing their blastomere content by extending the prefreeze culture time.*

***Methods:*** *Of a total of 3480 Day 2 embryos studied, 1921 (55.2%) had reached the 4-cell stage by 40 h postinsemination (FAST) and were transferred or cryopreserved. The remaining embryos that underwent subsequent cell division by 46 h (INTERMEDIATE; 18.3% of total) or 66 h (SLOW; 20.3% of total) were also cryopreserved whereas the 6.2% that remained arrested at 66 h were discarded. Thawed embryos from each category were assessed for survival, postthaw cleavage, and implantation.*

***Results:*** *The proportion of thawed embryos that survived, the proportion of surviving embryos that underwent postthaw cleavage, and the implantation rate of transferred embryos were all reduced in the slower growing cryopreserved embryos.*

***Conclusions:*** *The growth rate, and not the number of blastomeres per se, is a critical factor in predicting the developmental potential of cryopreserved embryos.*

**KEY WORDS:** Blastomere number; cryosurvival; embryo cryopreservation; embryo growth rate; implantation rate.

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## INTRODUCTION

The routine use of embryo cryopreservation in conjunction with clinical in vitro fertilization (IVF) is of crucial importance in optimizing the pregnancy potential while minimizing the risks associated with multiple pregnancy in cycles that generate multiple embryos (1–3). Predictors of the developmental potential of frozen embryos are of critical value in determining optimal strategies for their efficient utilization. For example, we have shown that Day 2 (postinsemination) embryos that survive cryopreservation without the loss of blastomeres have similar developmental potential to equivalent fresh embryos whereas blastomere loss is directly related to loss of viability (4). This analysis also demonstrated that fully intact, thawed cleavage-stage embryos that consisted of only two blastomeres when frozen on Day 2 implanted at a significantly lower rate than equivalent thawed 4-cell embryos did. This increase in implantation potential as a function of more advanced cleavage stage was consistent with our observations on otherwise matched nonfrozen embryos (4) and previous work (5), which suggested that cleavage stage may be more important than minor fragmentation in determining the developmental potential of IVF embryos.

Whether the relationship between cleavage stage and implantation potential is related to the number of blastomeres per se or whether growth rate is a marker of intrinsic embryonic viability cannot be adequately resolved by studies on fresh embryos because the two parameters are inextricably linked. Resolution of this question could have important implications in strategies for embryo cryopreservation if the blastomere number proves to be the important determinant. If this were the case, extending the prefreeze culture


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period for slower cleaving embryos in order to allow them to be frozen at the same cleavage stage as faster embryos could improve the outcome from cryopreserved embryos.

In the present study, we have investigated the outcome following the cryopreservation of embryos with differing cleavage rates at an equivalent cell stage.

## MATERIALS AND METHODS

### Embryos

The embryos included in the present study were generated during 1998 and 1999 using ovarian stimulation, in vitro fertilization, and embryo culture methodology, which has been described previously (4,6). Only embryos with ≤30% cytoplasmic fragmentation were considered suitable for cryopreservation and included in the study.

A total of 3480 embryos were assessed approximately 40 h after insemination. Of these embryos, 1921 (55.2%) had reached the 4-cell stage and were either transferred or cryopreserved. Cryopreserved embryos from this group were classified as FAST. Slower growing embryos (351) were transferred in cases where no other embryos were available for transfer, leaving 1208 embryos that were incubated for an extended period and were reassessed at 46 h and 66 h. Of these, 494 (40.9%) had undergone further cleavage at 46 h, were classified as INTERMEDIATE, and were also frozen. A further 547 (45.3%) had undergone further cleavage by 66 h, were classified as SLOW, and were also frozen. The remaining 167 (13.8%) had arrested at the 2-cell stage and were classified as DISCARDED.

Thus, the FAST, INTERMEDIATE, SLOW, and DISCARDED groups represented 55.2, 18.3, 20.3, and 6.2% respectively of the total population of embryos.

### Cryopreservation, Thawing, and Transfer

Embryo cryopreservation, using 1,2-propanediol and sucrose as cryoprotectants (7), thawing, and transfer were carried out as previously described (4) except that embryos were thawed on the day prior to transfer, cultured overnight (18–24 h), and assessed for further development.

### Analysis of Results

Blastomere loss and overnight cleavage were assessed for all thawed embryos from each of the three categories. Implantation rates (IR; fetal heart beats per 100 embryos transferred) were calculated from embryo transfer procedures in which embryos only from the same category (i.e., FAST, INTERMEDIATE, or SLOW) were transferred. Proportions were compared using the $\chi^2$ test.

## RESULTS

### Embryo Survival as a Function of Prefreeze Growth Rate

Having allowed all embryos to complete a second cleavage division prior to cryopreservation, the embryos were subsequently assesssed for postthaw blastomere survival according to the time at which they reached this stage. As can be seen from the results shown in Table I, there was a trend toward poorer survival in the slower growing embryos with significantly fewer completely intact embryos and significantly more embryos with <50% blastomere survival in the SLOW group.

### Postthaw Development as a Function of Prefreeze Growth Rate

Table II shows the frequency of postthaw cleavage in embryos that completed the second cleavage division at different times prior to cryopreservation. Further cell division was significantly more prevalent in embryos that had displayed more rapid development prior to freezing. This trend was evident when all embryos with 50% or more blastomere survival were assessed and also when the analysis was restricted to embryos that were completely intact after thawing (Table II).

### Implantation of Thawed Embryos as a Function of Prefreeze Growth Rate

The results in Table III demonstrate a trend toward a higher implantation rate of thawed embryos

**Table I.** Blastomere Survival in Relation to Prefreeze Growth Rate After Thawing of Cryopreserved FAST ($n = 588$), INTERMEDIATE ($n = 281$), and SLOW ($n = 293$) Embryos

| Blastomere survival (%) | FAST | INTERMEDIATE | SLOW |
|---|---|---|---|
| 100 | 313 (53.2%) | 153 (54.5%) | 116 (39.6%)** |
| 50–99 | 162 (27.6%) | 57 (20.3%)* | 82 (28.0%) |
| 0–49 | 113 (19.2%) | 59 (25.3%) | 95 (32.4%)*** |

* $p < .05$; ** $p < .01$; *** $p < .001$ relative to FAST embryos.

**Table II.** Proportion of Embryos Undergoing Postthaw Cleavage in Relation to Prefreeze Growth Rate and Extent of Blastomere Survival

| Blastomere survival (%) | FAST | INTERMEDIATE | SLOW |
|---|---|---|---|
| 100 | 188/313 (64.2%) | 51/153 (33.3%)** | 25/116 (21.6%)** |
| 50–99 | 61/162 (45.7%) | 13/57 (22.8%) | 15/82 (18.3%)* |
| 50–100 | 249/475 (52.4%) | 64/210 (30.5%)** | 40/198 (20.2%)** |

* $p < 1.05$; ** $p < .001$ relative to FAST embryos.

(including partially intact surviving embryos) with increasing prefreeze growth rate. In order to determine unequivocally the origin of an implantation event, only those embryo transfer procedures that contained embryos of equivalent prefreeze growth characteristics were included in this analysis.

## DISCUSSION

A clear relationship between cleavage rate during early preimplantation stages in vitro and successful embryogenesis has previously been demonstrated in hamster embryos by McKeirnan and Bavister (8) who emphasized the importance of considering both time and stage of development, rather than stage alone, when attempting to predict subsequent viability. Although this principle has been incorporated routinely in the assessment of human embryos prior to transfer in clinical IVF for many years (9), it is only recently that the relative importance of growth rate has been unequivocally established for human embryos (4,5). The study of McKiernan and Bavister (8) further demonstrated that additional time in culture to permit slower hamster embryos to catch up did not eliminate their developmental handicap. The possibility of allowing a "catch up" period for slower embryos in human IVF does not exist for fresh embryos because of considerations of synchrony between the embryo and the recipient endometrium, but it is possible to introduce such a period for embryos that are destined for cryopreservation. The present study has examined the impact of freezing embryos at an equivalent cell stage (4 blastomeres) on the postthaw characteristics and subsequent implantation of embryos with different growth rates.

The extent of blastomere survival is an important determinant of viability in cryopreserved embryos (4,10). Our results show a clear decrease in the survival of slow growing embryos relative to fast growing embryos frozen at an equivalent cell stage. The underlying cause of this differential cryosurvival is not clear because our historical data on overall blastomere survival in Day 2 embryos shows no significant difference between 2-cell and 4-cell embryos, in agreement with the values that can be calculated from previously published data (11).

Similarly, resumption of mitosis following thawing, another parameter associated with successful implantation (12,13), was less frequent in embryos that required an extended period to complete the second cleavage division prior to cryopreservation. This relationship was also observed to be independent of blastomere survival.

Finally, the implantation rate of slow embryos was observed to be reduced relative to that of faster embryos. The reduction in survival and the consequently lower proportion of slow embryos that would be available for transfer results in a ratio of more than 2:1 in the expected yield of FHs per embryo thawed in the SLOW versus the FAST embryos in this study (9.2%:4.0% = 2.3). This ratio is similar to the values obtained when the implantation rates of fresh or fully intact thawed Day 2 embryos with 4 versus 2 blastomeres are compared (4), suggesting that the extended time in culture has not enhanced the relative developmental potential of the cryopreserved slower growing embryos.

**Table III.** Implantation Rates of Thawed Embryos in Relation to Prefreeze Growth Rate[a]

| | FAST | INTERMEDIATE | SLOW |
|---|---|---|---|
| No. of embryos transferred | 362 | 129 | 128 |
| No. of FHs detected | 36 | 10 | 7 |
| Implantation rate | 10.0% | 7.8% | 5.5% |

[a] Analysis includes only embryo transfer procedures involving thawed embryos with equivalent prefreeze growth rates but includes both fully and partially intact embryos.

In conclusion, our results suggest that developmental potential is an inherent characteristic of individual embryos, which is reflected in cleavage rate and which cannot be altered by manipulating blastomere number via extended time in culture. Although strategies for embryo utilization in IVF may employ extended culture to assist selection of embryos for fresh transfer (14), the use of such an approach to allow a "catch up" period in conjunction with cryopreservation would not appear to offer any significant advantage.

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