Human blastocyst morphological quality is significantly improved in embryos classified as fast on day 3 ($\geq$10 cells), bringing into question current embryological dogma

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Objective: To evaluate developmental potential of fast cleaving day 3 embryos.
Design: Retrospective analysis.
Setting: Academic reproductive center.
Patient(s): Three thousand five hundred twenty-nine embryos.
Intervention(s): Day 3 embryos were classified according to cell number: slow cleaving: $\leq$6 cells, intermediate cleaving: 7–9 cells, and fast cleaving: $\geq$10 cells, and further evaluated on day 5. The preimplantation genetic diagnosis (PGD) results of 43 fast cleaving embryos were correlated to blastocyst formation. Clinical outcomes of transfers involving only fast cleaving embryos (n = 4) were evaluated.
Main Outcome Measure(s): Blastocyst morphology correlated to day 3 blastomere number. Relationship between euploidy and blastocyst formation of fast cleaving embryos. Implantation, pregnancy (PR), and birth rates resulting from fast embryo transfers.
Result(s): Blastocyst formation rate was significantly greater in the intermediate cleaving (72.7%) and fast cleaving (54.2%) groups when compared to the slow cleaving group (38%). Highest quality blastocysts were formed significantly more often in the fast cleaving group. Twenty fast cleaving embryos that underwent PGD, formed blastocysts, of which 45% (9/20) were diagnosed as euploid. Aneuploidy was diagnosed in 82.6% (19/23) of arrested embryos. A 50% implantation and 100% PR and birth rate were achieved with embryo transfers involving fast cleaving embryos.
Conclusion(s): Fast cleaving embryos not only reach the blastocyst stage at a similar rate to intermediate cleaving embryos, but also exceed morphological quality criteria on day 5. Fast cleaving embryo transfers demonstrated a high clinical potential. (Fertil Steril® 2008;89:358–63. ©2008 by American Society for Reproductive Medicine.)

Key Words: Fast cleaving embryos, blastocyst morphology, blastomere number

One of the major challenges in clinical assisted reproduction has been the ability to accurately select those embryos that are most likely to implant and give rise to a clinical pregnancy. Retrospective analyses have demonstrated that certain morphological criteria (e.g., blastomere symmetry, absence of fragmentation) correlate with higher implantation rates, whereas other morphological findings (e.g., multinucleated blastomeres, slow cleavage) correlate with lower rates of implantation (1, 2). The IVF laboratories often consider embryos that are developing faster on day 3 ($\geq$10 cells, $\sim$72 hours after retrieval) to be abnormal. It has been suggested that these fast embryos have significantly decreased developmental ability and may in fact have chromosomal abnormalities or abnormal epigenetic imprinting that may lead to their not initiating compaction and activation of the embryonic genome at the correct developmental stage (3, 4).

An association between early cleaving embryos and improved developmental potential has been described since 1973 in mouse embryos (5). In bovine embryos, early first cleavage has been correlated with a higher rate of blastocyst formation (6, 7). Other animal studies (rhesus monkey and sheep) have also shown that faster cleaving embryos can lead to significantly increased birth rates (8, 9).

In human reproduction, some investigators assess embryo quality by focusing on timely achievement of early developmental milestones as predictors of implantation potential (cleavage stage transfers), whereas other embryologists have used the advent of high quality sequential extended culture conditions to determine the optimal embryos for transfer (blastocysts transfers). Numerous reports have identified early embryo cleavage (24–27 hours after insemination) as a strong predictor of positive outcomes (10–12). Other

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human studies have been seemingly contradictory, suggesting that fast embryos showed poor pregnancy rates (PR) (13). Early cleavage on day 1 has been linked to high levels of polyspermic fertilization and possible mosaicism (14). Furthermore, in one study from 1998, in which chromosomal analysis of embryos was performed (for chromosomes X, Y, 13, 16, 18, and 21), approximately 70% of fast cleaving embryos were found to be chromosomally abnormal (15). These findings cannot be extrapolated to blastocyst formation as many chromosomally abnormal embryos have been shown to arrest before developing to the blastocyst stage by some of these same investigators (16).

We set out to determine the inherent developmental potential of fast cleaving day 3 embryos by evaluating and correlating the number of blastomeres identified on day 3 of culture with the developmental stage reached on day 5. In addition, to determine the developmental potential of embryos, we evaluated the expansion grade, inner cell mass (ICM), and trophectoderm quality of the blastocysts formed based on the day 3 cell number observed. Clinical outcome was evaluated in those patients that had embryo transfers with blastocysts that had developed from embryos classified as ≥ 10 cells on day 3 of culture. Finally, chromosomal analysis of embryos with ≥ 10 cells on day 3 was evaluated in IVF cases that had preimplantation genetic diagnosis (PGD) performed.

MATERIALS AND METHODS

This is a retrospective data analysis of embryology records at a large academic reproductive center during a 12-month period (January 1, 2005 to December 31, 2005). Institutional Review Board (IRB) approval for retrospective data collection and analysis of laboratory and clinical data after removal of all patient-specific identifiers was obtained. A total of 7,562 day 3 embryos were evaluated at approximately 72 hours after oocyte retrieval by light microscopy (magnification, $\times$400). Embryos were classified into three groups based on the number of cells visualized: slow cleaving embryos—≤ 6 cells; intermediate cleaving embryos—7–9 cells; and fast cleaving embryos—≥ 10 cells. The embryos from assisted reproductive technology (ART) cases that were not transferred on day 3 (n = 3,529) were further evaluated by light microscopy (magnification, $\times$300) at approximately 120 hours after retrieval, and their respective developmental stage reached on day 5 (arrested, morula, or blastocyst) was recorded. In addition, embryos that formed blastocysts were graded for degree of expansion of the blastocoel cavity, quality of the ICM, and the trophectoderm score by using a modified grading system based on the classifications originally reported by Gardner and Schoolcraft (17). Blastocysts were given a numerical score from 1–6 on the basis of their degree of expansion and hatching status, as follows: 1, an early blastocyst with a blastocoel that is less than half of the volume of the embryo; 2, a blastocyst with a blastocoel that is half of or greater than half of the volume of the embryo; 3, a blastocyst with a blastocoel completely filling the embryo; 4, an expanded blastocyst with a blastocoel volume larger than that of the early embryo, with a thinning zona pellucida (ZP); 5, a hatching blastocyst with the trophectoderm starting to herniate through the ZP; or 6, a hatched blastocyst, in which the blastocyst has completely escaped from the ZP. For blastocysts with expansions graded as 3–6, the development of the ICM was assessed as follows: A, tightly packed, many cells; B, loosely grouped, several cells; C, few cells slightly disorganized; or D, very few disorganized and uneven cells. The trophectoderm of the blastocysts with expansion grades of 3–6 was assessed as follows: A, many cells forming a cohesive epithelium; B, few cells forming a loose epithelium; C, few large cells; or D, very few cells of an uneven nature.

Chromosomal analysis of at least five chromosomes (13, 18, 21, X, and Y) from one blastomere was carried out in a limited number of fast cleaving day 3 embryos from PGD cases. An analysis of the number and percentages of embryos with normal versus abnormal chromosomal complements and the developmental stages reached of these embryos was performed. Compilation of the clinical outcome measures from embryo transfers involving fast cleaving embryos was performed for implantation, PRs, and birth rates.

Data analysis was performed comparing the cell number of a day 3 embryo to its development on day 5, blastocoel expansion, as well as its ICM and trophectoderm grading. Embryos classified as 4AA or greater were also correlated to their observed development on day 3 of culture. Statistical analysis was performed with χ^2 and Spearman's correlation coefficient for nonparametric data. Significance was set at $P<.05$.

RESULTS

On day 5 of culture, the 3,529 embryos were classified as follows: 872 (24.7%) arrested, 421 (11.9%) reached the morula stage, and 2,236 (63.4%) developed to blastocysts. Blastocyst formation rates for each one of the day 3 embryo groups (slow, intermediate, and fast cleaving) are included in Table 1. The mean number of blastomeres in the slow cleavage group was 5.20 ($\pm$0.81), whereas in the intermediate cleavage group, the mean number of cells was 7.78 ($\pm$0.59) and in the fast cleavage group the mean blastomere number was 11.19 ($\pm$1.88). The rate of blastocyst development was significantly greater in the intermediate (72.7%) and the fast (54.2%) cleaving groups when compared to the slow group (38%) ($P<.0001$). The rate of embryo arrest was significantly different between the three groups, with the intermediate group having the fewest embryo arrests ($P<.001$).

The number and percentage of blastocysts with each expansion grade, ICM, and trophectoderm scores were classified according to the number of cells that were present on day 3 (Table 2). A significant difference in blastocyst development between the slow, intermediate, and fast cleavage embryos was demonstrated across all groups for blastocyst expansion ($P<.001$), ICM quality ($P<.0001$), and trophectoderm grades ($P<.0001$). These findings show a direct correlation between embryo cleavage rates and the highest quality

TABLE 1

Embryo developmental stage reached on day 5 classified according to day 3 cell number.

	Slow cleavage (≤6 cells)	Intermediate cleavage (7–9 cells)	Fast cleavage (≥10 cells)
Blastocysts	315 (38%)	1804 (72.7%)	117 (54.2%)
Morula	141 (17%)	271 (10.9%)	9 (4.2%)
Arrested	374 (45.1%)	408 (16.4%)	90 (41.7%)

Note: $P<.0001$.

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blastocyst development with embryos considered fast cleaving on day 3 creating the greatest percentage of embryos classified as having grade 5 expansion, grade A ICM, and grade A trophectoderm scores (Figs. 1 to 3).

In addition, the analysis demonstrated that embryos in the fast cleaving group had a significantly greater likelihood of reaching grade 4AA or 5AA blastocysts ($P<.0001$). These high quality blastocysts were produced 12.8% of the time from the fast cleaving embryos, 7.0% of the time from the intermediate embryos, and only 0.6% of the time from the slow cleaving embryos (Table 3).

Given the retrospective nature of this study, and current bias against transfer of fast cleaving embryos, only four patients had blastocyst transfers in which all of the embryos transferred had been classified as fast on day 3. A total of eight blastocysts were transferred to the four patients, with a resulting implantation rate of 50%, and both the clinical PRs and live birth rates being 100%. In 25 additional patients, who underwent a blastocyst transfer, in which at least one of the embryos was classified as fast on day 3, a total of 26 fast embryos were transferred. The implantation rate in these mixed transfer cycles was 60% (24/69). The clinical PR was 60% (15/25) and live birth rate was 44% (11/25). The loss rate reported in this mixed transfer group of patients was 26.7% (4/15).

The PGD analysis on 43 fast cleaving embryos was performed with 20 (46.5%) of these embryos forming blastocysts, of which 45% (9/20) were diagnosed as euploid. Of those biopsied embryos that did not form blastocysts ($n = 23$), aneuploidy was diagnosed in 82.6% (19/23). Statistical significance was not reached, although a trend was found ($P = .10$), when comparing euploidy rates between those embryos that reached blastocysts and those that did not.

DISCUSSION

In human embryology, prevailing dogma states that on day 3 of culture, 7–9 blastomeres represent the optimal number of cells for normal embryos. In fact, in many IVF centers, faster developing embryos are generally not chosen for embryo transfer because of the concern that fast embryos are suboptimal, and may not develop normally. Analysis of a large series of embryos tracked from day 3 to day 5 in vitro suggests that the dogma describing poor prognosis of rapidly cleaving day 3 embryos may not be completely descriptive of their actual potential to form high quality blastocysts when using extended sequential culture techniques.

In our center, the average cell number on day 3 of embryo development is 7–9 cells. We used this information to classify day 3 embryos into three groups—slow, intermediate, and fast. Embryos with ≤6 cells were classified as slow cleaving, whereas embryos with ≥10 cells were considered as having fast development. Our results demonstrate that the majority of embryos classified as fast on day 3 reach the blastocyst stage. In addition, these fast cleaving embryos form the most desired high quality blastocysts (4AA or better) in comparison to the other day 3 embryo cleavage groups ($P<.001$).

Edwards et al. (18) were the first to recognize that the faster cleaving embryos were more likely to give rise to a pregnancy than slower cleaving embryos. It has since become common to consider developmental stage and morphological appearance at the time of transfer as predictors of implantation potential (7). Shapiro et al. (19) suggested that embryos that grew to 8 or more cells on day 3 in fact produced blastocysts nearly 76% of the time. On the other hand, in that study, many of the embryos growing at a significantly slower rate (5 cells on day 3 did not form blastocysts, 87%). An evident relationship between the time of first cleavage after insemination and the developmental competence of the embryos has been demonstrated in many species (rhesus monkey,1983; hamster, 1994; buffalo, 1996; mouse, 1998; and cattle, 1999), with those oocytes cleaving earliest being more likely to reach the blastocyst stage than their later-cleaving counterparts (5, 8, 20–22). In humans, patients whose embryos cleaved to the 8-cell stage by 55 hours after insemination achieved a PR of nearly double than that for embryos that reached the same stage of development after 55 hours in culture (19). Subsequently, many reports have confirmed the usefulness of this 8-cell developmental phenomenon in selecting human embryos with improved developmental competence (8, 10, 23, 24). Faster developing cleavage stage embryos have been reported to result in higher PRs after both single and multiple embryo transfers (25). All of these previous findings demonstrate that early cellular cleavage and the

TABLE 2

Day 5 Blastocyst grading classified according to day 3 cell number observations.

	Blastocyst grading according to day 3 cell number		
	Slow cleavage	**Intermediate cleavage**	**Fast cleavage**
Expansion			
1	76	185	5
	24.13%	10.25%	4.27%
2	76	236	9
	24.13%	13.08%	7.69%
3	86	478	23
	27.30%	26.50%	19.66%
4	54	539	30
	17.14%	29.88%	25.64%
5	23	365	50
	7.30%	20.23%	42.74%
6	0	1	0
	0%	0.06%	0%
			$P<.001$
Inner cell mass			
A	16	377	33
	11.11%	28.84%	36.26%
B	59	517	38
	40.92%	39.56%	41.76%
C	63	399	19
	43.75%	30.53%	20.88%
D	6	14	1
	4.17%	1.07%	1.1%
			$P<.0001$
Trophectoderm			
A	11	302	30
	6.83%	21.85%	28.85%
B	54	568	36
	33.54%	41.1%	34.62%
C	68	419	23
	42.24%	30.32%	22.12%
D	28	93	15
	17.39%	6.73%	14.42%
			$P<.0001$

Luna. Fast cleaving embryos form high quality blastocysts. Fertil Steril 2008.

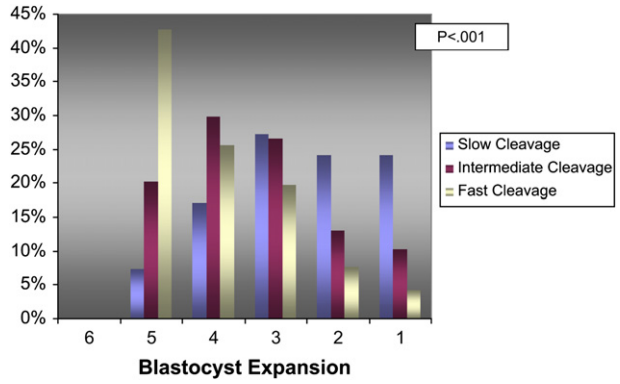
developmental rate at which an embryo reaches the 8-cell stage are important measurements for future development and successful outcomes.

To our knowledge this is the first analysis that describes that fast cleaving day 3 embryos ($\geq$10 cells) not only reach the blastocyst stage at a similar percentage to intermediate cleaving embryos (7–9 cells), but that the blastocyst morphology reached on day 5 of culture from these fast embryos exceeds the quality of embryos that present only an intermediate number of cells on day 3. The finding that the expanded blastocysts (grades 4 and 5) were formed most often by embryos in the fast group (68.4%) versus the intermediate group (50.1%) demonstrates that these fast embryos should be considered normal from the standpoint of potential for blastocyst development. In fact, our data suggest that the embryos classified as fast on day 3 may be the most optimal embryos from a standpoint of day 5 blastocyst developmental expansion and most highly desired ICM and trophectoderm grades.

As a result of these findings we believe that the dogma currently espoused, that fast cleaving embryos on day 3 ($\geq$10 cells) are abnormal and cannot develop, may be false. In fact, our results suggest that these embryos are the ones that look the best after another 48 hours of incubation in extended sequential culture systems.

FIGURE 1

Blastocyst expansion grade classified according to embryo cell number on day 3 of culture.



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Given our findings, we can conclude that embryos showing a fast cleavage rate on day 3 can, and in fact, do result in higher quality blastocysts, based on morphology reached on day 5 of development. The fast cleaving embryo group had a significantly greater degree of blastocoel expansion than embryos considered to have an intermediate cleavage rate on day 3, and the fast embryos also developed to blastocysts with higher quality ICM and trophectoderm grades than the slow or intermediate cleaving embryo groups. We recognize the potential criticisms of this study, which includes its retrospective nature and that many of the embryos from the fast developing group were not chosen for embryo transfer. Although very limited data were available from embryo transfers with all fast cleaving embryos (n = 4), we did achieve a 50% implantation rate and 100% clinical PRs and live birth rates for these cycles.

FIGURE 2

Blastocyst inner cell mass (ICM) grading classified according to day 3 embryo cell number.

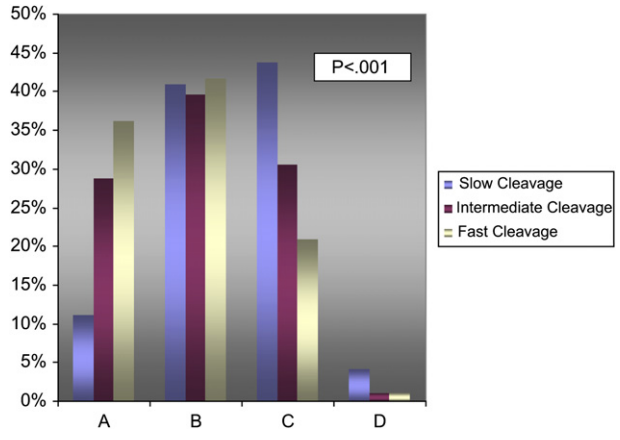

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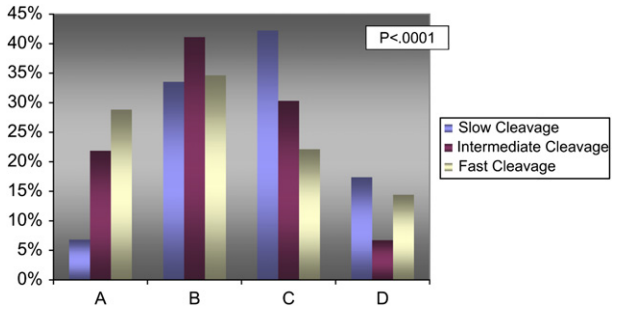
FIGURE 3

Blastocyst trophectoderm grade classified according to day 3 embryo cleavage group.



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Therefore, the clinical outcome potential of fast cleaving embryos has been established to be quite high.

In the very limited number of cases in which fast cleaving embryos were biopsied for PGD analysis on day 3 of culture, our findings demonstrate that the rate of blastocyst development is similar to those fast embryos that did not undergo a blastomere biopsy. Although not significant, the embryos that did form blastocysts had a nearly three times higher euploidy rate than those that did not.

Although this initial study analyzed specifically the correlation between day 3 cell number and blastocyst developmental potential, further analysis of the overall effects of cellular fragmentation and multinucleation of the day 3 embryo and their relationship to blastocyst development is currently ongoing. Further analysis of the chromosomal complement of these blastocysts is also underway, including both ICM and trophectodermal cells. The previous publications demonstrating the limited ability of chromosomally abnormal embryos to form high quality blastocysts gives us hope that we will not see levels of chromosomal abnormalities as high as those previously reported at the day 3 developmental stage, and we may find that if abnormalities do exist that they are confined to only the extraembryonic tissues.

Our analysis of more than 3,000 individually cultured embryos suggests that the number of blastomeres identified on day 3 of culture can predict the rate of blastulation and resultant quality of the blastocysts produced. Further analysis is now underway to better identify the ultimate reproductive competence of these embryos as measured by euploidy and implantation potential. In addition, further advances in embryonic fingerprinting to correlate embryonic outcome in multiembryo transfers and analysis of single embryo transfer cases with the blastocysts developing from the intermediate versus the fast cleaving day 3 embryos could both improve our understanding of the correlation between day 3 embryonic cell number and ultimate pregnancy outcome. If these fast embryos are better than their slower counterparts, then

TABLE 3

High quality blastocyst formation rates according to day 3 cleavage group.

	Slow cleavage	Intermediate cleavage	Fast cleavage
Blastocyst 4AA or 5AA	2/315 (0.63%)	127/1804 (7.03%)	15/117 (12.82%)

Note: $P < .0001$.

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it may be time to modify current embryological thinking and to begin to transfer these fast cleaving embryos.

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