Early cleavage morphology affects the quality and implantation potential of day 3 embryos

Haydar Nadir Çiray, M.D., Ph.D., Levent Karagenç, Ph.D., Ulun Uluğ, M.D., Faruk Bener, M.D., and Mustafa Bahçeci, M.D.

Bahceci Women Health Care Center and German Hospital, Istanbul, Turkey

Objective: To assess the development and implantation potential of early-cleaved embryos displaying various morphological patterns.
Design: Retrospective analysis.
Setting: Private IVF center.
Patient(s): Embryos obtained from 1,556 transfer cycles were assessed. Early-cleaved embryos were grouped according to their cleavage patterns as: even (1,490); uneven (3,238); and fragmented (768), or according to nuclear morphologies as: mononucleation (2,008) and other nuclear morphologies; nonmononucleation (3,488). Seven thousand four hundred forty-five embryos were late cleaved.
Intervention(s): None.
Main Outcome Measure(s): Embryo quality, pregnancy (PR), and implantation rates.
Result(s): Day3 embryo quality was highest in evenly early-cleaved embryos and in those displaying mononucleation. Early-cleaved embryos displaying fragmentation and late-cleaved embryos yielded the poorest day 3 quality. Early cleavage cycles displayed higher PR and implantation rate than late cleavage with the exception of other nuclear morphologies, in which similar outcome was obtained. Mononucleated early-cleaved embryos implanted at a higher frequency than early-cleaved embryos displaying other nuclear morphologies.
Conclusion(s): The morphology of early cleavage correlates to day 3 embryo quality and implantation rate. (Fertil Steril® 2006;85:358–65. ©2006 by American Society for Reproductive Medicine.)

Key Words: Early cleavage, mononucleation, embryo quality, implantation

Identification of an embryo with the highest implantation potential is of fundamental importance in assisted reproduction. In recent years, the rate of mitosis gained importance to evaluate development and implantation potential of embryos (1). One indicator of mitotic rate is the time of initial cleavage of the zygote. Several studies have shown that appearance of a two-cell embryo between 25 and 27 hours after insemination (defined as early-cleaved embryos) yielded better quality embryos with higher implantation potential (2–8) and develop to blastocysts at a higher frequency (9) compared to late-cleaved zygotes.

Early cleavage can be observed in various morphologies. These involve variations in cleavage patterns and in appearance of blastomere nuclei. With regard to cleavage pattern, blastomeres may appear evenly (symmetrical) or unevenly sized with or without presence of fragments (asymmetrical). The nuclei of corresponding blastomeres in an early-cleaved embryo may be invisible or visible displaying mononucleation or multinucleation, or have a complex appearance displaying combination of these morphologies. Therefore, there is a range of embryos that can be defined as early cleaved.

Received February 12, 2005; revised and accepted July 19, 2005.
Reprint requests: Mustafa Bahçeci, M.D., Azer Is Merkezi 44/17 Kat 6, Abdi Ipekci Cad., Nisantasi 80200, Istanbul, Turkey (FAX: 90-2122303990; E-mail: mbahceci@superonline.com).

Only few early cleavage studies described the assessed morphological pattern, which were exclusively the cleavage pattern. Lundin et al. (8) reported results from early-cleaved embryos of identical cleavage score. Fisch et al. (10) introduced blastomere symmetry and degree of fragmentation of the early-cleaved embryos as a parameter to predict blastocyst formation. We previously defined early cleavage as "cleavage without fragments" and presented data accordingly (6). Other studies (2–5, 7) have not described the morphology of early cleavage.

To our knowledge the nuclear morphology of early-cleaved embryos has never been studied. It has been shown that day 2 embryos displaying multinucleated blastomeres have a reduced implantation potential (11–15). A recent study proposed nuclear morphology to be more important than conventional parameters based on blastomere size and clearness and degree of fragmentation in selection of transfers (16).

Whether embryos formed from various early cleavage morphologies possess similar implantation potential is not known. The present study tests the hypothesis that early cleavage morphology affects the quality and implantation potential of day 3 embryos. Therefore, the cleavage pattern (even, uneven, and fragmented) and nuclear morphology (mononucleation and nonmononucleation) of early-cleaved embryos were correlated to day 3 embryo quality, pregnancy (PR), and implantation rates and were compared to those

0015-0282/06/$32.00

doi:10.1016/j.fertnstert.2005.07.1301

obtained from late cleaved embryos. The findings of the present study are expected to contribute to the definition of early cleavage, and may therefore aid recognizing embryos with high implantation potential at the initial cleavage. Such information may be useful for centers where embryo selection for transfer is performed at early stages of development.

MATERIALS AND METHODS

The hypothesis of the study was tested on embryo transfer cycles that have been performed under a period of 7 months in the Assisted Conception Unit of the German Hospital in Istanbul. Data was analyzed retrospectively. All couples gave informed consent, and the ethics committee of the hospital approved the study protocol. Embryos obtained from women less than 38 years of age were taken into evaluation. Only first trial cycles were accepted to the survey. The infertility etiologies of the cycles were as follows: male factor infertility, tubal, uterine, diminished ovarian reserve, polycystic ovary syndrome (PCOS), unexplained, or combined. The distribution of these etiologies was similar between groups.

Pituitary desensitization with a GnRH analogue and ovarian stimulation with gonadotropins were carried out as described previously (17). A long protocol regime has been used in all cycles. Oocytes were recovered 35 hours after hCG injection. The embryology procedures of our laboratory have been described in detail earlier (6). All oocytes underwent intracytoplasmic sperm injection (ICSI), as this was the investigators' universal practice. Fertilization was inspected 16–18 hours after ICSI, and two pronuclei oocytes were cultured individually in 30-μL droplets of G1.3 medium (Vitrolife, Gothenburg, Sweden) covered with mineral oil.

Early cleavage inspection was performed on the heated stage of an inverted microscope equipped with Hoffman optics (Zeiss, Axiovert 135M, Gottingen, Germany) at exactly 26 hours after termination of ICSI. Embryos displaying two cells at inspection were considered as early cleaved, and others as late cleaved. Embryos exhibiting more than two cells, which constituted less than 0.05 % of all cleavages, were excluded from the study. Two embryologists inspected early cleavage morphology. The information that an embryo was early cleaved was accessible to all other laboratory personnel, but morphology was not. Two parameters of early-cleaved embryos were independently assessed: cleavage pattern and nuclear morphology. Two-cell embryos with similar blastomere sizes, as visually determined at ×400 magnification of the inverted microscope, were defined as "even cleavages" (Fig. 1C,D). Other cleavages without fragments were grouped as "uneven cleavages" (Fig. 1A,E). Cleavages with fragments were considered as "fragmented" (Fig. 1B,F). The nuclear morphology of early-cleaved embryos was concomitantly inspected and evaluated independently from the cleavage pattern. Embryos exhibiting single nuclei in both blastomeres were defined as mononucleated (Fig. 1C), whereas the remainder of the two-cell embryos were defined as nonmononucleated (Fig. 1A,B,D–F).

Embryos were subsequently inspected at day 3 (66–68 hours after ICSI). Embryo quality was calculated by multiplying the morphological grade (1 to 4 grading system of Bolton et al. (18) in which grade 4 is morphologically the best) with the number of blastomeres (19). To avoid bias in the study, selection of transfer embryos was performed in the absence of the embryologists who assessed early cleavage morphology. Embryos with highest qualities were transferred and when embryos displayed similar qualities, those showing early cleavage were chosen. All transfers were performed at day 3 using an Edwards-Wallace (Smiths Medical Int'l. Ltd., Kent, UK) catheter under ultrasound guidance.

Statistics

Statistical analysis was carried out using GraphPad InStat 3 software (Graph Pad Inc., San Diego, CA). Differences between groups were analyzed by analysis of variance followed by appropriate post-hoc tests (Tukey-Kramer), where applicable. The χ^2 and Fisher's exact tests were used to compare differences between PR and implantation rate. A P value less than .05 were considered statistically significant.

RESULTS

Effects of Early Cleavage Morphology on Day 3 Embryo Quality

Embryos obtained from 1,556 transfer cycles were assessed. The characteristics of these cycles are given in Table 1. A total of 13,412 zygotes were obtained of which 12,941 (96.5%) cleaved. From 12,941 embryos, 5,496 (42.5%) were early and 7,445 (57.5%) were late cleaved.

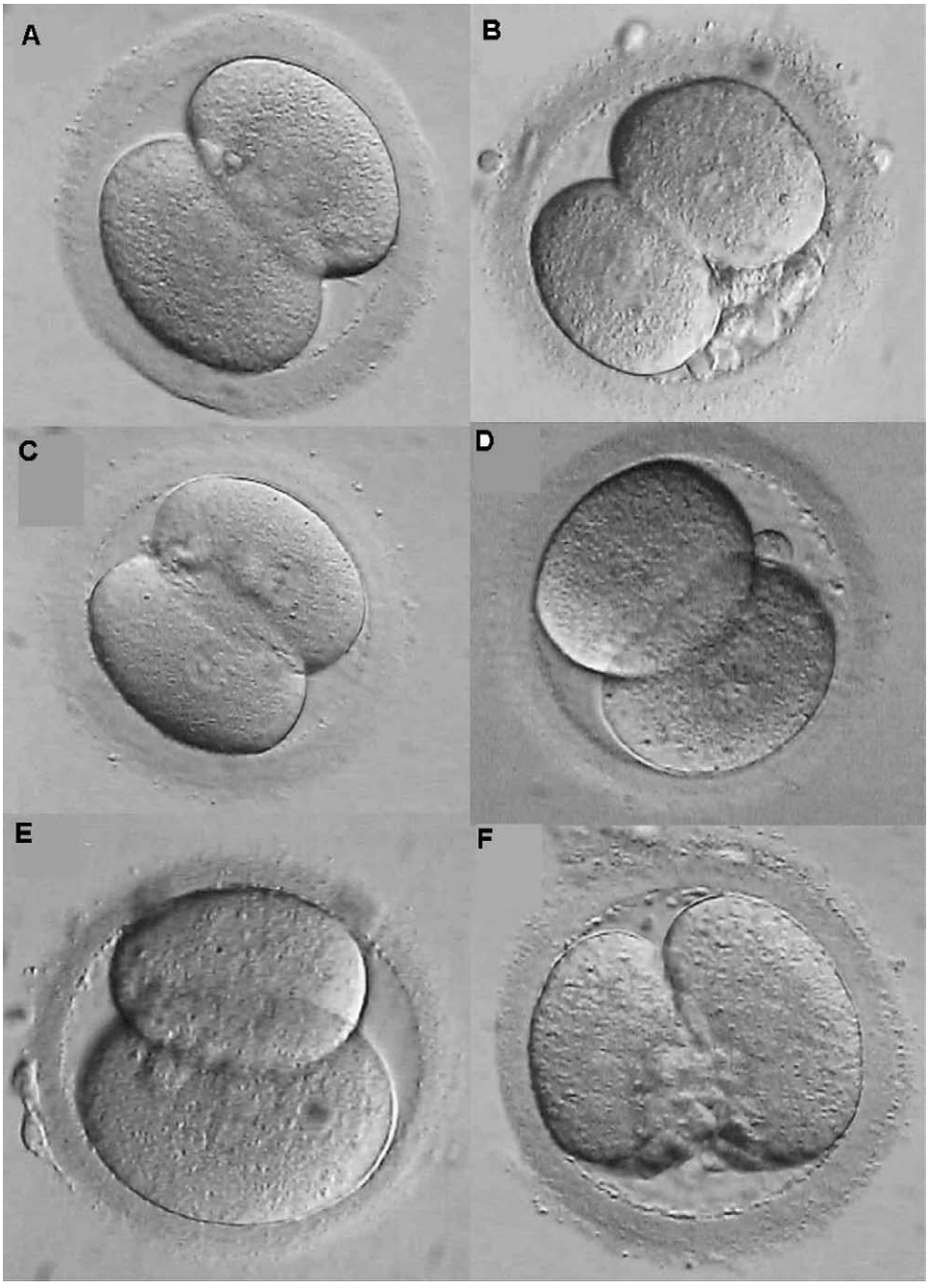
From 5,496 early-cleaved embryos, 1,490 (27.1%) cleaved evenly, 3,238 unevenly (58.9%), and 768 cleaved with fragments (14.0%). The 2,008 early-cleaved embryos displayed mononucleation (36.5%), and 3,488 showed other nuclear morphologies (63.5%). Day 3 embryo quality differed between groups ($P<.0001$; Table 2). Evenly cleaved embryos displayed the highest day 3 score among all groups ($P<.001$). Mononucleated early cleavages displayed a lower ($P<.001$) score than evenly cleaved embryos, which was higher ($P<.001$) than all other groups. Uneven cleavages and other nuclear morphologies had a similar score, which was higher ($P<.001$) than fragmented early-cleaved embryos and late cleavages, but lower ($P<.001$) than evenly cleaved embryos and mononucleated early cleavages. Fragmented early cleavages and late cleavages displayed a similar day 3 score, which was the poorest ($P<.001$) among all groups.

Effects of Early Cleavage Morphology on Pregnancy and Implantation Rates

To evaluate the effects of early cleavage morphology on PR and implantation rate, 1,556 cycles were grouped into those in which all transfer embryos derived from a similar early

FIGURE 1

Examples of early cleavage morphologies. Cleavage patterns of these embryos are uneven (**A and E**), even (**C and D**), fragmented (**B and F**), and nuclear morphologies are mononucleation (**C**), invisible (**A, E, and F**), and asymmetry (**B and D**). Embryos displaying invisible and asymmetrical nuclear morphologies are classified as nonmononucleated.



Çiray. Early cleavage morphology and implantation. Fertil Steril 2006.

cleavage morphology or late cleavage (cycles with homogenous transfers). The outcome measures of these cycles are presented in Table 3. In 30 cycles transfer embryos formed from evenly (even early cleavage cycles), and in 75, from unevenly early-cleaved zygotes (uneven early cleavage cycles). There was one fragmented early cleavage cycle and it was not taken into statistical evaluation. Transfer embryos of 100 cycles originated from mononucleated early-cleaved zygotes (mononucleated early cleavage cycles) and in 50, from early-cleaved zygotes displaying other nuclear mor-

TABLE 1

The characteristics of cycles assessed in the present study.

Characteristics	Data
No. of cycles	1,556
Mean age of women ± SD (range)	31.3 ± 4.3 (18–38)
Mean day 3 FSH ± SD (range)	6.8 ± 3.5 (0.01–28)
Mean ampules consumed ± SD (range)	39.7 ± 18.4 (0–151)
Mean peak E_2 level ± SD (range)	2,826 ± 1,325 (225–8,720)
Mean oocytes retrieved ± SD (range)	14.4 ± 8.1 (1–78)
Mean MII oocytes ± SD (range)	11.8 ± 6.9 (1–66)
Mean fertilized (2 PN) oocytes ± SD (range)	8.6 ± 5.7 (1–57)
Total no. of fertilized (2 PN) oocytes	13,412
No. of cleaved embryos (% fertilized oocytes)	12,941 (96.5)
Early-cleaved embryos (% cleaved embryos)	5,496 (42.5)
Late-cleaved embryos (% cleaved embryos)	7,445 (57.5)

Çiray. Early cleavage morphology and implantation. Fertil Steril 2006.

phologies (nonmononucleated cycles; others). In 187 cycles, none of the transfer embryos were early cleaved (late cleavage cycles).

Late cleavage cycles displayed poorer characteristics than early cleavage regarding mean age of women, mean number of ampules consumed, mean peak E_2 value, mean number of collected oocytes, mean ratio of MII oocytes, and mean fertilization rate. The characteristics of early cleavage cycles remained similar between various cleavage morphologies.

The mean number of transfer embryos differed between groups ($P<.0001$). Even early cleavages displayed the lowest and late cleavage cycles displayed the highest number of transfers among all groups. The mean score of transfer embryos also differed between groups ($P<.0001$). The lowest mean embryo score has been observed in late cleavage cycles among all groups ($P<.001$). Early cleavage cycles displaying other nuclear morphologies showed a lower mean embryo score than even early cleavage cycles ($P<.001$) and those displaying mononucleation ($P<.05$).

The PR and implantation rate differed between groups ($P=.001$ and $P<.0001$, respectively). Early cleavage cycles, with the exception of other nuclear morphologies, displayed higher PR and implantation rate than late cleavage, in which similar rates were obtained. There was no difference in the PR between early cleavage cycles displaying various morphologies. The implantation rate of early cleavage cycles with mononucleation was higher than other nuclear morphologies ($P=.04$).

DISCUSSION

Several studies reported that early cleavage yielded better embryo quality, and their transfer resulted at higher PR and implantation rate compared to late-cleaved embryos (2–8). Early cleavage, regardless of morphology, yielded better quality embryos when compared to late cleavage in the present study, with the exception of those displaying fragments. This finding is in accordance to our earlier study (6) in which two-cell embryos displaying fragments cleaved to poor quality day 3 embryos, and therefore, have not been considered as early cleaved. Although the outcome of fragmented early cleavage cycles have not been assessed, similar results to late cleavage could be expected due to similar day 3 embryo qualities. The variation among early-cleaved embryos, on the other hand, received little attention and to our knowledge, the present is the first study to classify and assess early cleavage according to cleavage pattern and nuclear morphology. The findings have shown that the morphology of early cleavage affected development and implantation potential of embryos.

The present study used day 3 quality as the main transfer embryo selection criteria. Early-cleaved embryos have been preferred for transfer only when day 3 qualities were similar. The superiority of this selection policy over the traditional system, which is based merely on embryo morphology, has recently been shown (5). In the present study the quality of transfer embryos was correlated with the implantation rate. Transfer of embryos derived from evenly early-cleaved zygotes and those displaying mononucleation yielded higher implantation rates compared to other nuclear morphologies. Although day 3 qualities of unevenly early-cleaved embryos were lower than even cleavages and those displaying mononucleation (Table 2), transfer embryos were of similar quality (Table 3), hence, a similar implantation rate has been obtained between these groups.

The impact of cleavage pattern on the implantation potential of embryos could be predicted if the events or mechanisms that determine the time and pattern of initial cleavage was understood. It has been suggested that oocytes with a "high metabolic fitness" cleave earlier due to the availability

TABLE 2

Day 3 embryo scores correlated to early cleavage patterns and late cleavage.

Early-cleaved embryos	
Cleavage pattern	
Even	
No. of embryos (% early-cleaved embryos)	1,490 (27.1)
Mean day 3 score ± SD (range)	23.6 ± 5.7 (12–36)[a]
No. of homogeneous ET cycles	30
Uneven	
No. of embryos (% early-cleaved embryos)	3,238 (58.9)
Mean day 3 score ± SD (range)	20.4 ± 5.3 (6–32)[b]
No. of homogeneous ET cycles	75
Fragmented	
No. of embryos (% early-cleaved embryos)	768 (14.0)
Mean day 3 score ± SD (range)	16.1 ± 6.2 (3–36)[c]
No. of homogeneous ET cycles	1[e]
Nuclear morphology	
Mononucleated	
No. of embryos (% early-cleaved embryos)	2,008 (36.5)
Mean day 3 score ± SD (range)	22.2 ± 5.0 (6–33)[c]
No. of homogeneous ET cycles	100
Other nuclear morphologies	
No. of embryos (% early-cleaved embryos)	3,488 (63.5)
Mean day 3 score ± SD (range)	20.7 ± 5.7 (4–38)
No. of homogeneous ET cycles	50
Late-cleaved embryos	
No. of embryos	7,445
Mean day 3 score ± SD (range)	15.9 ± 4.7 (3–32)[d]
No. of homogeneous ET cycles	187

[a] $P<.001$ vs. uneven, fragmented, mono and others.
[b] $P<.001$ vs. fragmented and mono.
[c] $P<.001$ vs. others.
[d] $P<.001$ vs. even, uneven, mononucleated, and others.
[e] Not considered for statistical evaluation.

Çiray. Early cleavage morphology and implantation. Fertil Steril 2006.

and competence of ATP, mRNA, mitochondria, and so on (8). The first mitotic division distributes the components that are laid down in the oocyte during development to two blastomeres and disruption of the cytoplasmic rotation after fertilization may result in unequal and incorrect distribution of certain gene products or cytoplasmic components to the cells, which will be perpetuated during development (20). We are tempted to speculate that early cleavage and symmetry represent two distinct features, indicative of the concentration and distribution of nuclear and cytoplasmic components. Therefore, an embryo with a high concentration of intracellular components may be expected to cleave early, regardless of symmetry, but when the distribution is not homogenous between blastomeres (asymmetry), further development of the embryo and thus, implantation, may be compromised. As a result, an asymmetrically early-cleaved embryo may have a low implantation potential compared to symmetrical cleavage.

In the present study, early cleavage displaying mononucleation yielded higher embryo quality and implantation rate than other nuclear forms, indicating the importance of nuclear morphology at the initial cleavage. The variations in nuclear morphology of blastomeres and their effects in predicting the success rates has been studied extensively in day 2 embryos (11–15, 21–25). The normality of blastomere nuclei has recently been suggested as a more important index of quality than conventional fragmentation features or blastomere uniformity analysis when choosing transfer embryos (16).

The blastomeres of normal human embryos are mononucleated (26). Embryos with multinucleated blastomeres yield lower implantation rates (24) from those displaying mononucleation (12, 13, 15, 16). Multinucleation has been shown to correlate to aneuploidy and to uneven blastomere size (22), and these embryos arrested development or caused complex chromosomal abnormalities when implanted (23).

TABLE 3

Characteristics and the outcome of cycles in which transfer embryos were derived from early cleaved zygotes displaying even or uneven blastomeres, mononucleation, nonmononucleation, and late cleavage (mean ± SD).

	Cleavage pattern		Nuclear morphology		Late cleavage	
	Even	Uneven	Mono	Others		
No. of cycles	30	75	100	50	187	
Mean age of women	28.6 ± 4.3	29.7 ± 4.0	29.9 ± 4.1	29.7 ± 4.7	31.2 ± 4.0[a]	.002
(range)	(20–37)	(21–38)	(20–38)	(21–37)	(19–38)	
Mean day 3 FSH	6.8 ± 4.0	6.1 ± 2.1	6.2 ± 2.3	5.9 ± 3.1	6.6 ± 3.3	NS
(range)	(0.5–14.5)	(2.2–10.3)	(0.4–11.1)	(0.5–14.5)	(0.01–16)	
Mean ampoules consumed	29.5 ± 11.1	28.8 ± 11.9	30.9 ± 12.7	31.1 ± 12.7	39.8 ± 16.9[b]	<.0001
(75IU/amp) (range)	(13–54)	(13–84)	(12.5–64)	(7.0–84)	(12–112)	
Mean peak E_2 value	3325 ± 1343	3476 ± 1116	3431 ± 1472	3063 ± 1148	2828 ± 1225[c]	.0002
(range)	(489–6134)	(1698–7168)	(489–7478)	(334–6288)	(734–6940)	
Mean collected oocytes	20.3 ± 10.3	18.1 ± 8.7	18.4 ± 8.4	17.1 ± 6.8	14.2 ± 6.5[d]	<.0001
(range)	(4–48)	(4–55)	(4–48)	(4–35)	(2–36)	
% MII/collected oocytes	84.9 ± 12.4	83.0 ± 13.6	84.8 ± 12.5	86.2 ± 10.8	80.1 ± 13.8[e]	.007
% Fertilization rate	76.1 ± 14.1	76.7 ± 15.2	78.8 ± 12.1	75.3 ± 16.3	72.6 ± 17.9[f]	.03
Mean transferred embryos	2.1 ± 0.3[g]	2.5 ± 0.6	2.5 ± 0.5	2.5 ± 0.5	2.9 ± 0.6[h]	<.0001
(range)	(2–3)	(2–4)	(2–4)	(2–3)	(2–5)	
Mean embryo score	27.7 ± 3.6	25.2 ± 3.4	25.7 ± 3.4	23.6 ± 4.8[i]	20.2 ± 5.1[j]	<.0001
(no. of transferred embryos)	(64)	(184)	(251)	(126)	(545)	
Implantation (%)	32 (50.0)	76 (41.3)	118 (47.0)	45 (35.7)[k]	148 (27.2)[l]	<.0001[n]
Pregnancy (%)	21 (70.0)	49 (65.3)	72 (72.0)	32 (64.0)	93 (49.7)[m]	.001[o]

[a] $P<.05$ vs. even.
[b] $P<.01$ vs. even and others, $P<.001$ versus uneven and mono.
[c] $P<.01$ vs. uneven and mono.
[d] $P<.01$ vs. uneven, and $P<.001$ versus even and mono.
[e] $P<.05$ vs. mono and others.
[f] $P<.05$ vs. mono.
[g] $P<.01$ vs. uneven and mono, $P<.05$ vs. others.
[h] $P<.001$ vs. even, uneven, mono, and others.
[i] $P<.001$ vs. even, $P<.05$ vs. mono.
[j] $P<.001$ vs. even, uneven, mono, and others.
[k] $P=.04$ vs. mono, OR 1.60, 95% CI 1.03–2.48, $P=0.06$ versus even.
[l] $P=.0003$ vs. even, OR 2.68, 95% CI 1.59–4.54, $P=.0004$ vs. uneven, OR 1.52, 95% CI 1.22–1.90, $P<.0001$ vs. mono, OR 2.38, 95% CI 1.74–3.25.
[m] $P=.04$ vs. even, OR 2.36, 95% CI 1.03–5.42, $P=.03$ vs. uneven, OR 1.91, 95% CI 1.09–3.32, $P=.0003$ vs. mono, OR 0.38, 95% CI 0.23–0.65.
[n] χ^2 39.48, DF 4.
[o] χ^2 16.71, DF 4.

Çiray. Early cleavage morphology and implantation. Fertil Steril 2006.

In the present study, nonmononucleation has been combined into one group, and included a number of morphologies, such as invisible nuclei, multinucleation, or combination of these appearances in respective of blastomeres. Individual assessment of these nuclear morphologies could yield embryos with different development and implantation capacities. However, assessment of nonmononucleated early cleavage is difficult. Embryos with invisible nuclei at the time of inspection (prophase cells) constitute a mixed group, as blastomeres may be mononucleated or multinucleated, or combination of these morphologies. Similarly, observation of multinucleation may be confusing, unless at least three nuclei are seen in a single blastomere, as new nuclear membranes are formed around each set of the two sets of 46 chromosomes before cytokinesis (telophase of mitosis). Furthermore, early-cleaved embryos displaying different nuclear morphologies may indicate asymmetrical or asynchronical cleavage. Accordingly, day 2 embryos displaying multinucleation in one or more blastomeres have been shown to possess compromised genetic quality in the corresponding blastomeres (15). Moriwaki et al. (16) showed that embryos displaying mononucleation in some blastomeres implant at a lower rate compared to those showing mononucleation in all cells.

The high implantation rate obtained after transfer of embryos derived from mononucleated early-cleaved zygotes may indicate that mechanisms causing mononucleated day 2 embryos implant at a high incidence and initiate from the first cleavage. It has been reported that two-thirds of multinucleated blastomeres have defective cleavage in a mononucleated parent cell, such as flawed chromosome migration, nuclear fragmentation, failure to divide, or errors in cell packaging (25). Hardy (27) reported that multinucleation compromises implantation in early cleavage stages, but not at the eight-cell and later stages. The results of the present study are further evidence of the importance of mononucleation in predicting the implantation potential of an embryo at early stages of development. The development and implantation capacity of nonmononucleated early-cleaved embryos remains to be investigated in detail. Well-defined nuclear morphologies accompanied by a high number of homogenous transfer cycles are required for proper analysis of such an investigation.

In conclusion, according the findings of the present study, we suggest that when early cleavage is taken as a parameter for selection of transfers, cleavages displaying even blastomeres or mononucleation should be chosen when possible. Fragmented cleavages should not be considered as early cleaved. Definition of early cleavage morphology strengthened the importance of this parameter in reducing multiple PR at early stage transfers. Further studies are required in which early cleavage mechanisms that contribute to the development of the early embryo.

REFERENCES

1. Edwards RG, Fishel SB, Cohen J, Fehilly CB, Purdy JM, Slater JM, et al. Factors influencing the success of in vitro fertilization for alleviating human infertility. J In Vitro Fert Embryo Transf 1984;1:3–23.
2. Sakkas D, Shoukir Y, Chardonnens D, Bianchi PG, Campana A. Early cleavage of human embryos to the two-cell stage after intracytoplasmic sperm injection as an indicator of embryo viability. Hum Reprod 1998;13:182–7.
3. Bos-Mikich A, Mattos AL, Ferrari AN. Early cleavage of human embryos: an effective method for predicting successful IVF/ICSI outcome. Hum Reprod 2001;16:2658–61.
4. Salumets A, Hyden-Granskog C, Makinen S, Suikkari AM, Tiitinen A, Tuuri T. Early cleavage predicts the viability of human embryos in elective single embryo transfer procedures. Hum Reprod 2003;18:821–5.
5. Van Montfoort AP, Dumoulin JC, Kester AD, Evers JL. Early cleavage is a valuable addition to existing embryo selection parameters: a study using single embryo transfers. Hum Reprod 2004;19:2103–8.
6. Ciray HN, Ulug U, Bahceci M. Transfer of early-cleaved embryos increases implantation rate in patients undergoing ovarian stimulation and ICSI-embryo transfer. Reprod Biomed Online 2004;8:219–23.
7. Wharf E, Dimitrakopoulos A, Khalaf Y, Pickering S. Early embryo development is an indicator of implantation potential. Reprod Biomed Online 2004;8:212–8.
8. Lundin K, Bergh C, Hardarson T. Early embryo cleavage is a strong indicator of embryo quality in human IVF. Hum Reprod 2001;16: 2652–7.
9. Neuber E, Rinaudo P, Trimarchi JR, Sakkas D. Sequential assessment of individually cultured human embryos as an indicator of subsequent good quality blastocyst development. Hum Reprod 2003;18:1307–12.
10. Fisch JD, Rodriguez H, Ross R, Overby G, Sher G. The Graduated Embryo Score (GES) predicts blastocyst formation and pregnancy rate from cleavage-stage embryos. Hum Reprod 2001;16:1970–5.
11. Jackson KV, Ginsburg ES, Hornstein MD, Rein MS, Clarke RN. Multinucleation in normally fertilized embryos is associated with an accelerated ovulation induction response and lower implantation and pregnancy rates in in vitro fertilization–embryo transfer cycles. Fertil Steril 1998;70:60–6.
12. Gerris J, De Neubourg D, Mangelschots K, Van Royen E, Van de Meerssche M, Valkenburg M. Prevention of twin pregnancy after in-vitro fertilization or intracytoplasmic sperm injection based on strict embryo criteria: a prospective randomized clinical trial. Hum Reprod 1999;14:2581–7.
13. Gerris J, De Neubourg D, Mangelschots K, Van Royen E, Vercruyssen M, Barudy-Vasquez J, et al. Elective single day 3 embryo transfer halves the twinning rate without decrease in the ongoing pregnancy rate of an IVF/ICSI programme. Hum Reprod 2002;17:2626–31.
14. Van Royen E, Mangelschots K, De Neubourg D, Valkenburg M, Van de Meerssche M, Ryckaert G, et al. Characterization of a top quality embryo, a step towards single-embryo transfer. Hum Reprod 1999;14: 2345–9.
15. Van Royen E, Mangelschots K, Vercruyssen M, De Neubourg D, Valkenburg M, Ryckaert G, et al. Multinucleation in cleavage stage embryos. Hum Reprod 2003;18:1062–9.
16. Moriwaki T, Suganuma N, Hayakawa M, Hibi H, Katsumata Y, Oguchi H, et al. Embryo evaluation by analysing blastomere nuclei. Hum Reprod 2004;19:152–6.
17. Ulug U, Jozwiak EA, Mesut A, Berksoy MM, Bahceci M. Survival rates during the first trimester of multiple gestations achieved by ICSI: a report of 1448 consecutive multiples. Hum Reprod 2004;19:360–4.
18. Bolton VN, Hawes SM, Taylor CT, Parsons JH. Development of spare human preimplantation embryos in vitro: an analysis of the correlations among gross morphology, cleavage rates, and development to the blastocyst. J In Vitro Fert Embryo Transf 1989;6:30–5.
19. Steer CV, Mills CL, Tan SL, Campbell S, Edwards RG. The cumulative embryo score: a predictive embryo scoring technique to select the optimal number of embryos to transfer in an in-vitro fertilization and embryo transfer programme. Hum Reprod 1992;7:117–9.

20. Van Blerkom J, Davis P, Alexander S. Differential mitochondrial distribution in human pronuclear embryos leads to disproportionate inheritance between blastomeres: relationship to microtubular organization, ATP content and competence. Hum Reprod 2000;15:2621–33.
21. Alikani M, Calderon G, Tomkin G, Garrisi J, Kokot M, Cohen J. Cleavage anomalies in early human embryos and survival after prolonged culture in-vitro. Hum Reprod 2000;15:2634–43.
22. Hardarson T, Hanson C, Sjogren A, Lundin K. Human embryos with unevenly sized blastomeres have lower pregnancy and implantation rates: indications for aneuploidy and multinucleation. Hum Reprod 2001;16:313–8.
23. Magli MC, Gianaroli L, Ferraretti AP. Chromosomal abnormalities in embryos. Mol Cell Endocrinol 2001;183 Suppl 1:S29–34.
24. Pelinck MJ, De Vos M, Dekens M, Van der Elst J, De Sutter P, Dhont M. Embryos cultured in vitro with multinucleated blastomeres have poor implantation potential in human in-vitro fertilization and intracytoplasmic sperm injection. Hum Reprod 1998;13:960–3.
25. Pickering SJ, Taylor A, Johnson MH, Braude PR. An analysis of multinucleated blastomere formation in human embryos. Hum Reprod 1995;10:1912–22.
26. Staessen C, Van Steirteghem A. The genetic constitution of multinuclear blastomeres and their derivative daughter blastomeres. Hum Reprod 1998;13:1625–31.
27. Hardy K. Cell death in the mammalian blastocyst. Mol Hum Reprod 1997;3:919–25.