REPRODUCTIVE BIOLOGY

Impact of early cleaved zygote morphology on embryo development and in vitro fertilization–embryo transfer outcome: a prospective study

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Objective: To evaluate the impact of the first division morphology on embryo development and IVF–embryo transfer outcome.
Design: Prospective study.
Setting: Teaching hospital, France.
Patient(s): All zygotes from 201 couples were checked for early cleavage. We defined as "even," early cleaved (EC) zygotes with 2 cells of even size; as "uneven," EC zygotes with 2 cells of uneven size; and as "fragmented," EC zygotes with more than 20% fragmentation rate. Day 2 embryo quality was assessed as "top" embryo or "non-top," with the evaluation of multinucleated blastomeres.
Intervention(s): None.
Main Outcome Measure(s): Day 2 embryo quality, pregnancy and implantation rates.
Result(s): Among EC zygotes, 59.1% were even, 13.0% were uneven, and 27.9% were fragmented. Even EC yielded more "top" embryos and less multinucleated blastomere embryos than uneven EC (77.0% vs. 46.3%) and fragmented EC (77.0% vs. 13.9%). The 125 double embryo transfers that comprised at least one embryo derived from even EC zygote led to higher pregnancy rate (PR) (64.0% vs. 43.4%) and implantation rate (42.0% vs. 27.6%) compared to the 76 double embryo transfers with embryos derived from breakdown or 2PN zygotes.
Conclusion(s): The morphology of the early cleaved zygote is involved in embryo development. Evaluation of this morphology is an effective and valuable method of assessing the embryo quality. (Fertil Steril® 2007;■:■-■. ©2007 by American Society for Reproductive Medicine.)
Key Words: Early cleavage, embryo development, embryo fragmentation, first cleavage plane

Embryo implantation is the main limiting factor in the success rate of IVF–embryo transfer. In assisted reproductive technology (ART) laboratories, several approaches to identify the best embryos for embryo transfer have been carried out with the aim to reduce the number of transferred embryos. Attributes of the embryo are well described in the literature. First, cleavage speed, whether too slow or too fast is considered to have a negative impact on implantation rate (1–4). Second, cellular cleavage, when uneven, seems to negatively affect the developmental capacity of the embryo (2, 3, 5). Third, the extent of fragmentation has been reported to have a predictive value on embryo potential implantation rate by several in-

vestigators (2, 3, 5–8). Early morphological data have also been examined and results indicate that their assessment can add substantially to the selection of the embryo with the highest implantation potential. Among them, zygote morphological parameters have been proposed, such as number of nucleolar precursor bodies, their distribution in the pronuclei, and the presence or absence of a cytoplasmic halo (9–14).

More recently, the timing of the first cleavage of the zygote has been proven useful for the selection of embryos with the highest implantation potential. Transfer of embryos coming from early cleaved zygotes are associated with higher pregnancy rate (PR) and implantation rate compared to the transfer of non early cleaved embryos (15–23).

However, early cleaved zygotes appear to be an heterogeneous population, morphology wise, depending on the quality of the first mitotic division. A typical meridional axis divides the ooplasm into two equal halves, sometimes with

slight differences in the size of the two daughter cells (24–28). On the contrary, this first cleavage, when irregular, produces an embryo with two unequal blastomeres or fragmentation. Ciray et al. (29) focused on the morphological aspects of early cleaved zygotes, and suggested, in a retrospective study, that early cleaved zygotes displaying even blastomeres, yielded the best day 3 embryo quality.

Consequently, the hypothesis that morphology of early cleaved zygote might influence embryo quality and ART outcome could be postulated. We have conducted a prospective study, focusing on the impact of the first mitotic division morphology on the early embryo development and IVF–embryo transfer outcome.

MATERIALS AND METHODS
Patients

From February to December 2005, we studied prospectively 201 IVF–embryo transfer attempts for 201 couples. All women met the following inclusion criteria: normal ovarian reserve on day 3 (basal FSH level and antral follicular count), adequate response to controlled ovarian hyperstimulation (COH) (at least four mature follicles on trigger day), and were less than 42 years old.

Indications for IVF–embryo transfer were tubal factor (29.3%), male factor infertility (40.8%), endometriosis (9.0%), anovulatory (2.5%), or unexplained infertility (18.4%). This prospective study received approval of our internal Institutional Review Board (IRB).

Controlled Ovarian Hyperstimulation Protocol

Women received a time-release GnRH agonist (GnRH-a) (3 mg IM, Decapeptyl; Beaufour Ipsen Pharma, Paris, France) on cycle day 2. Three weeks later, complete pituitary desensitization was confirmed by the detection of low serum E_2 and gonadotropin levels. Patients also had a conventional ultrasound examination to exclude ovarian cysts and to verify that endometrial thickness was <5 mm. Combined recombinant FSH (225 IU/day, SC) and LH (75 IU/day, SC) therapy (Gonal-F and Luveris, respectively; Serono Pharmaceuticals, Boulogne, France) was then initiated. Daily FSH and LH doses and timing of hCG administration were further adjusted according to the usual criteria of follicular maturation. Administration of hCG (10,000 IU, IM, Gonadotrophine Chorionique "Endo"; Organon Pharmaceuticals, Saint-Denis, France) was performed when at least four follicles exceeded 17 mm in diameter and E_2 levels per mature follicle (≥ 17 mm in diameter) were higher than 300 pg/mL. Oocytes were retrieved 36 hours after hCG administration by transvaginal ultrasound-guided aspiration. All embryo transfers were performed 2 days after oocyte retrieval using classic Frydman catheters (CCD Laboratories, Paris, France). Luteal phase was supported with micronized P (400 mg/day, Estima; Effik Pharmaceuticals, Bièvres, France) administered daily by vaginal route starting on the evening of embryo transfer.

Fertilization and Embryo Culture

Oocytes were rinsed and preserved in 3 mL of IVF culture medium (MediCult, Lyon, France) until sperm preparation. According to usual sperm parameters, as described elsewhere (30), we performed conventional IVF or intracytoplasmic sperm injection (ICSI) when necessary.

Fertilization was determined by confirmation of the 2PN and polar bodies 17–19 hours after microinjection/insemination. After fertilization, zygotes were cultured in microdrops of 35 µL ISM1 culture medium (MediCult) under oil at 37°C under 5% CO_2 until the second day of embryo development. All zygotes that were normally fertilized (2PN) were checked again in the afternoon of day 1 for early embryo cleavage within 25–26 hours after microinjection or 26–27 hours after insemination. A time difference in early cleavage assessment was necessary between IVF- and ICSI-derived embryos, to compensate for the time difference in their early development (31). Each zygote was observed by two individuals on the heated stage of an inverted microscope TE 300 equipped with a camera (Nikon, Champigny sur Marne, France). Three groups were defined: persistence of 2PN (2PN), 2PN breakdown (BD), and early cleaved zygotes (EC). The EC zygotes were rotated using a holding pipette to evaluate clearly their morphology. Number, respective size of cells, and percentage of anucleated fragment were recorded. The EC zygotes presenting with two cells of even size and less than 20% fragmentation were defined as "even EC zygotes." The EC zygotes with two cells of uneven size (difference >20%) and less than 20% fragmentation were scored as "uneven." The EC zygotes with more than 20% of fragmentation whatever the cells size, were defined as "fragmented."

Day 2 embryos were then graded (I, II, III, IV, V) on the basis of their morphology. Embryos with four mononucleated blastomeres of even size and no fragmentation were scored I. Those with four mononucleated blastomeres of even size and including 1%–10% fragmentation, or those showing appropriate developmental stage (3 or 5 blastomeres), were scored II. Embryos with four mononucleated blastomeres of even size with 11%–20% fragmentation were labeled III. Embryos with 21%–50% fragmentation, whatever the number of blastomeres or showing atypical developmental stage or those with at least one multinucleated blastomere, were scored IV. Those having more than 50% fragmentation were scored V. Embryos with grades I, II, and III were considered as top quality embryos.

Two hundred one double embryo transfers (DET) were performed on day 2 with the two embryos presenting with the highest quality at day 2. When more than two embryos displayed similar grade on day 2, those with early cleavage were chosen.

Statistics

The measure of central tendency used was the mean, and the measure of variability was the standard deviation. Confidence intervals (95% CI) were estimated when necessary.

Comparisons of the parametric variables were performed using a Fisher's test. Qualitative variables were compared using the χ^2 test.

All statistical tests were performed with the Statview 4.0 software (Abacus Concept Inc., Berkeley, CA). Test with a P value $<.05$ was considered statistically significant.

RESULTS

Incidence of Early Cleavage

A total of 1,307 zygotes were assessed for early cleavage: 39.5% were at the two-cell stage (EC), 31.1% were BD, and 29.4% were still at the 2PN stage.

Of 201 couples, 142 presented with an early cleavage (70.6%).

Impact of Early Cleavage Phenomenon on Day 2 Embryo Quality

Embryology data are shown in Table 1. As shown, EC zygotes yielded more top quality embryos than BD and 2PN zygotes. The EC zygotes yielded more top quality embryos than non-EC zygotes (55.4% vs. 37.7%, $P<.01$).

The prevalence of poor quality embryo (grade V) was significantly increased in the 2PN and BD zygotes compared to EC zygotes. Furthermore, embryos derived from 2PN and BD zygotes presented with more multinucleated blastomere than those derived from EC zygotes.

Impact of Early Cleaved Zygote Morphology on Day 2 Embryo Quality

Embryology data are shown in Table 2. Among the 516 EC zygotes, 59.1% were even, 13.0% were uneven, and 27.9% were fragmented. Day 2 embryo quality differed significantly between these three groups. Even EC zygotes yielded more "top" embryos than uneven and fragmented EC zygotes. Fragmented early cleaved zygotes displayed very poor day 2 embryo quality (34.7% grade V), similar to the 2PN group (33.5%).

Among EC zygotes, uneven and fragmented ones yielded significantly more embryos with multinucleated blastomere than even EC zygotes, similar to the 2PN and BD groups (19.5% and 16.5%, respectively).

Impact of Early Cleavage Phenomenon on Pregnancy and Implantation Rates

Among the 201 DET, 76 were performed with two embryos derived from EC zygotes, 49 had one embryo issued from EC zygotes, and 76 had only non-EC embryos. No difference was found between DET with one or two embryos derived from EC zygotes in terms of PR and implantation rate. Thus, two groups were defined: 125 DET with at least one embryo derived from EC zygotes (EC group) and the 76 DET with two embryos derived from non-EC zygotes (NEC group). Patients characteristics, IVF indications, and treatment regimen were comparable in the two groups (Table 3).

The ART outcomes are shown in Table 4. Both groups were comparable regarding the total number of retrieved oocytes, fertilization rates, and number of embryos obtained. The prevalence of top embryos obtained in the EC group was significantly higher than in the NEC group. As expected, the prevalence of top embryos transferred was also higher in the EC group. Clinical PRs (gestational sac with positive heart beat observed ultrasonographically at 7 weeks of amenorrhea) and ongoing PRs (>12 weeks of amenorrhea) per embryo transfer were statistically higher in the EC than in

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TABLE 1

Comparison of day 2 embryo development according to 2PN, BD, and EC zygotes.

Embryo grade D2	2PN N = 385	BD N = 406	EC N = 516	P value
I	5 (1.3%)	9 (2.2%)	32 (6.2%)	NS[a]
II	40 (10.4%)	79 (19.5%)	94 (18.2%)	NS
III	65 (16.9%)	100 (24.6%)	160 (31.0%)	NS
IV	146 (37.9%)	112 (27.6%)	164 (31.8%)	NS
V	129 (33.5%)[c]	106 (26.1%)[d]	66 (12.8%)	S[b]
I+II+III	110 (28.6%)[e]	188 (46.3%)[f]	286 (55.4%)	S
MNB	75 (19.5%)	67 (16.5%)	56 (10.9%)[g]	S

Note: 2PN = 2 pronuclei; BD = 2PN breakdown; EC = early cleaved zygote.
[a] Not significant.
[b] significant.
[c] $P<10^{-4}$ 2PN vs. EC.
[d] $P<10^{-4}$ BD vs. EC.
[e] $P<10^{-4}$ 2PN vs. BD and 2PN vs. EC.
[f] $P=.005$ BD vs. EC.
[g] $P<.001$ EC vs. 2PN and $P=.02$ EC vs. BD.

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TABLE 2

Comparison of day 2 embryo development according to different morphologies of early cleaved zygotes.

Embryo grade D2	Even N = 305	Uneven N = 67	Fragmented N = 144	*P* value
I	30 (9.8%)	2 (3.0%)	0	NS[a]
II	87 (28.5%)	6 (9.0%)	1 (0.7%)	NS
III	118 (38.7%)	23 (34.3%)	19 (13.2%)[b]	$<10^{-4}$
IV	64 (21.0%)[c]	26 (38.8%)	74 (51.4%)	$<10^{-4}$
V	6 (2.0%)	10 (14.9%)	50 (34.7%)[d]	$<10^{-4}$
I+II+III	235 (77.0%)[e]	31 (46.3%)[f]	20 (13.9%)	$<10^{-4}$
MNB	16 (5.2%)[g]	12 (17.9%)	28 (19.4%)	$<10^{-4}$

[a] Not significant.
[b] $P<10^{-4}$ even vs. fragmented and uneven vs. fragmented.
[c] $P<10^{-4}$ even vs. fragmented.
[d] $P<10^{-4}$ even vs. uneven and even vs. fragmented.
[e] $P<10^{-4}$ even vs. uneven and even vs. fragmented.
[f] $P<10^{-4}$ uneven vs. even and uneven vs. fragmented.
[g] $P<10^{-4}$ even vs. uneven and even vs. fragmented.

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the NEC group (80/125 vs. 33/76 and 73/125 vs. 31/76, respectively, *P*=.014 and .0027). Accordingly, implantation rates obtained in the EC and NEC groups were also statistically higher (105/250 vs. 42/152, *P*=.0067). Overall, of the 125 DET with at least one embryo derived from an EC zygote, we defined a subgroup of 62 DET for which embryo grading on day 2 was not sufficient for embryos selection. Thus, the early cleavage scoring was used as a discriminator (62/125, 49.6%). In this subgroup, we had an increase in the overall clinical, ongoing PR, and implantation rates, respectively 79.0% (49/62), 70.9% (44/62), and 54.8% (68/124), associated with a rate of twin pregnancy of 38.7% (19/49).

TABLE 3

76 double embryo transfers with embryos derived from non early cleaved zygotes (NEC group) and 125 double embryo transfers with at least one embryo derived from early cleaved zygotes.

	NEC	EC[a]	*P* value
Number of embryo transfers	76	125	
Age (years)	32.5 ± 4.1	32.1 ± 3.7	NS[b]
Basal FSH (IU/L)	6.1 ± 1.6	6.2 ± 1.5	NS
Antral follicular count	18.8 ± 7.8	18.4 ± 9.3	NS
Rank of IVF-embryo transfer attempt	2.1 ± 1.3	1.8 ± 1.0	NS
Male factor infertility	33 (43%)	49 (39%)	NS
Tubal factor	24 (31%)	35 (28%)	NS
Endometriosis	5 (7%)	13 (10%)	NS
Ovulatory	2 (3%)	3 (3%)	NS
Unexplained	12 (16%)	25 (20%)	NS
hMG requirement (IU)	2,656 ± 787	2,617 ± 755	NS
Day of hCG administration	11.9 ± 0.2	11.9 ± 0.2	NS
E_2 level (pg/mL)[c]	2,707 ± 985	2,731 ± 1,021	NS
Number of ≥17 mm follicles[c]	8.4 ± 2.9	8.1 ± 2.8	NS
Endometrial thickness (mm)[c]	9.2 ± 1.3	10.7 ± 1.4	NS
ICSI prevalence	51.3%	49.6%	NS

[a] EC zygotes were always even.
[b] Not significant.
[c] On the day of hCG administration.

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TABLE 4

IVF–embryo transfer outcome in the NEC and EC groups.

Embryo grade D2	NEC N = 76	EC[a] N = 125	P value
No. oocytes	11.3 ± 5.8	11.3 ± 4.5	NS[b]
No. 2PN	6.4 ± 3.6	6.5 ± 3.2	NS
Fertilization rates [95% CI]	66.7% [62.4–71.0]	70.6% [67.6–73.6]	NS
No. embryos obtained	6.8 ± 4.0	7.0 ± 3.2	NS
Prevalence of top quality embryos [95% CI]	41.6% [36.7–46.5]	52.2% [46.7–57.7]	.0068
Prevalence of top quality embryos transferred [95% CI]	72.4% [64.0–80.8]	83.2% [77.5–88.9]	.03
Clinical pregnancy rate per embryo transfer [95% CI]	43.4% [32.2–54.6]	64.0% [55.6–72.4]	.014
Ongoing pregnancy rate per embryo transfer [95% CI]	40.8% [29.7–51.9]	58.4% [49.7–67.1]	.0027
Implantation rate [95% CI]	27.6% [19.7–35.5]	42.0% [35.6–48.4]	.0067

Note: NEC = double embryo transfers with embryos derived from non early cleaved zygotes; EC = double embryo transfers with at least one embryo derived from early cleaved zygotes.

[a] EC zygotes were always even.

[b] Not significant.

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DISCUSSION

The present prospective study was designed to test the hypothesis that morphology of the first cleavage axis could influence embryo development and ART outcome. Its methodological characteristics included 201 DET. The selection of the transferred embryos was based first on their day 2 morphological aspect, and second, on their first cleavage morphology when day 2 morphology was not sufficient to discriminate transferred embryos.

Our results showed that EC zygotes, specifically even ones, yielded more top quality embryo than NEC zygotes. In addition, transfers of embryos derived from even EC zygotes resulted in overall significantly improved PR and implantation rate. Furthermore, for 62 DET, early cleavage was the discriminator factor used for embryo selection. We obtained 70.9% of ongoing pregnancy per embryo transfer (ET) but associated with a high rate of twin pregnancy (38.7%). These results permit us to identify a subgroup of patients for whom single embryo transfer (SET) must be proposed.

As shown in Table 5, several studies detailed the impact of the EC event on ART outcome. The benefit of early cleavage assessment was first put forth by Shoukir et al. (32) In a prospective study, these investigators observed a twofold increase of PR and a threefold increase of implantation rate in EC cycles compared to NEC cycles. These results were confirmed in two other prospective studies (16, 33). Another prospective study (18) was conducted on a larger size sample: 827 transfers for which the best embryos were chosen, irrespective of early cleavage. The researchers reported higher PR and implantation rate in the EC group as compared to the NEC group. Our data are in accordance with these previous results. We observed an implantation rate of 42.0% in DET when at least one EC zygote was replaced compared to 27.6% when the two transferred embryos derived from NEC zygotes (P<.01).

Furthermore, two studies were performed to evaluate the impact of the EC event on subsequent embryo development. They showed a positive correlation between EC zygotes, good quality four-cell embryos, and blastocyst development (20, 34). In agreement with these findings, we showed a significant increase in day 2 good quality embryos when derived from EC zygotes compared to non-EC ones (55.4% vs. 37.7%, P<.01).

However, in all of these previous studies, morphology of EC zygotes was not well described. This morphology can take several forms, which could affect embryo development. A detailed assessment of EC zygotes shows that first cleavage is not always symmetrical and could lead to irregular or fragmented two-cell embryo (29). This retrospective study showed that early cleavage morphology affects the quality and implantation potential of day 3 embryos. They reported a fourfold prevalence of uneven EC zygotes (3,238/5,496; 58.9%) compared to our findings (67/516; 13.0%). This discrepancy shows that EC morphology assessment remains subjective and has to be defined more precisely, in particular the selection of a critical size difference between the two blastomeres, which would be essential to score EC zygotes and discriminate subsequent day 2 embryos.

Ciray et al. (29), using a day 3 embryo score to evaluate embryo quality, showed that uneven EC zygotes yielded significantly lower quality embryos than even zygotes (P=.001), but surprisingly without impact on ART outcome. They performed 75 homogeneous transfers with embryos derived from uneven EC zygotes and 30 with embryos derived from even EC zygotes. The PR and implantation rate were similar in the two groups (65.3% vs. 70.0% and 41.3% vs. 50.0%, respectively). In our study, 76 homogeneous DET were performed with embryos derived only from even EC zygotes and the PR and implantation rate were 64.5% and 41.4%, respectively. No homogeneous DET was performed

TABLE 5

Early cleavage studies and IVF–embryo transfer in the NEC and EC groups.

	Shoukir et al., 1997	Sakkas et al., 1998	Sakkas et al., 2001	Lundin et al., 2001	Bos-Mikich et al., 2001
Design	Pospective	Prospective	Prospective	Prospective	Prospective
Cycles (n)	143	88	230	827	74
Assessed zygotes (n)	767	400	1,801	10,798	379
Assessment of EC morphology	No	No	No	No	No
% of day 2 good quality embryo NEC versus EC zygotes	NI	NI	NI	33.4 vs. 62.5 $P<.001$	NI
% of blastocystes NEC versus EC zygotes	–	–	–	–	–
Day of transfer	Day 2	Day 2	Day 2	Day 2/day 3	Day 3
Number of transferred embryos NEC group versus EC group (mean ±SD)	2.78 ± 0.7 vs. 2.67 ± 0.6	2.7 ± 0.8 vs. 2.8 ± 0.7	2.2 ± 0.6 vs. 2.2 ± 0.4	1.97 vs.1.92	6 ± 1.43 vs. 3.6 ± 1.1
Pregnancy rate (%) NEC group versus EC group	14.7 vs. 33.3 $P<.001$	5.9 vs. 25.9 $P<.05$	23.8 vs. 45.0 $P<.01$	31.3 vs. 40.5 $P<.01$	25.0 vs. 55.0 $P=.02$
Implantation rate (%) NEC group versus EC group	7.5 vs. 23.6 $P<.05$	3.2 vs. 14.0 $P<.01$	14.8 vs. 25.5 $P<.01$	19.5 vs. 28.0 $P<.001$	8.0 vs. 18.0 $P<.05$

Note: NEC = nonearly cleaved; EC = early cleaved; NI : not indicated.

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with embryos derived from uneven zygotes because of the availability of better quality embryos in all cases.

Furthermore, we showed in our study for the first time, a significant threefold increase of multinucleated blastomere in embryos derived from uneven zygotes compared to even ones (17.9% vs. 5.2%, $P=.00096$). Blastomere nuclear status has been shown to be a good indicator of embryo quality (35, 36). Possible mechanisms of multinucleation include karyokinesis in the absence of cytokinesis, partial fragmentation of the nuclei, or defective migration of chromosomes at the mitotic anaphase. This suggests that uneven first cleavage could be associated with an abnormal chromosomal status and additional studies are in progress to elucidate this mechanism, specifically in our preimplantation genetic diagnosis (PGD) program.

In the present study, our findings show that fragmentation involving a considerable portion (>20%) of the EC zygote, compromises the developmental potential of the embryo. Fragmented EC zygotes lead to a significant decrease of good quality day 2 embryos compared to non fragmented EC zygotes (13.9% vs. 77.0%, $P=2.10^{-5}$). The extent of fragmentation may disturb preimplantation development by selectively removing polarized molecules from specific regions of the cleaving zygote. Antczak and Van Blerkom (8) focused on the relationship between blastomere fragmentation and further developmental potential by defining specific patterns of fragmentation and examining the impor-

tance of the stage at which fragmentation occurs, and the particular blastomere involved. These investigators demonstrated that certain patterns of fragmentation can result in the partial or near total loss of regulatory proteins from specific blastomeres and thus embryo development can be compromised if it occurs during the two-cell stage.

Several studies have revealed the existence of an animal–vegetal axis in human oocytes and embryos (24). The animal pole may be estimated by the location of the first polar body, and after fertilization, the second polar body marks the so-called embryonic pole (26). In line with this, it has been suggested that proteins and gene products are not evenly distributed, but polarized in the zygote (8, 25). Because of oocyte and zygote polarity, it can be speculated that when the zygote divides unequally, the two daughter cells will receive unequal amounts of proteins, mRNA, and organites. This could induce a loss of essential molecules for one blastomere with a negative effect on subsequent cell divisions.

In this prospective study we have shown that the evaluation of EC morphology in an IVF program could be an effective and valuable method of assessing embryo viability. Our results indicate that early cleaved zygotes displaying even blastomeres and less than 20% fragmentation lead to an increase of good quality embryos. Given its ease of application, assessment of EC zygote morphology could be potentially used as an early discriminator that complements the day 2 embryo grading. The results of the present study offer

TABLE 5

Continued.

	Fenwick et al., 2002	Neuber et al., 2003	Warf et al., 2003	Van Montfoort et al., 2004	Ciray et al., 2006	Our study
	Prospective	Retrospective	Prospective	Retrospective	Retrospective	Prospective
	70	191	352	165	1,556	201
	579	1,550	2,447	945	13,412	1,307
	No	No	No	No	Yes	Yes
	NI	50 vs. 81 $P<.01$	30.8 vs. 82.3 $P<.01$	NI	NI	37.7 vs. 55.4 $P<.01$
	16.6 vs. 32.2 $P<.01$	39 vs. 21 $P<.01$	–	–	–	–
	Day 2	Day 5	Day 2	Day 2	Day 3	Day 2
	2.18 vs. 2.19	2.9 vs. 2.9	1.9 vs. 2.0	1.0 vs. 1.0	2.1 ± 0.3 vs. 2.9 ± 0.6	2.0 vs. 2.0
	10.5 vs. 31.3 $P<.05$	21.9 vs. 40.0 $P=.05$	17.2 vs. 43.2 $P<.001$	17.6 vs. 46.4 $P<.001$	49.7 vs. 66.6 $P<.001$	43.4 vs. 64.0 $P<.01$
	6.0 vs. 21.4 $P<.005$	14.3 vs. 26 $P=.03$	10.3 vs. 32.1 $P<.001$	17.6 vs. 46.4 $P<.001$	27.2 vs. 43.5 $P<.001$	27.6 vs. 42.0 $P<.01$

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evidence that early cleavage grading could have a critical impact on our daily practice by achieving embryos selection for transfer, to implement an elective SET policy.

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