Irregular cleavage of early preimplantation human embryos: characteristics of patients and pregnancy outcomes

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Abstract

Purpose This is a retrospective analysis of the morphokinetics, prevalence, and implantation potential of embryos with irregular first and second cleavages as identified by time-lapse microscopy.

Methods The study included 253 women who underwent 387 assisted reproduction treatments with intracytoplasmic sperm injection (ICSI). Each patient was assigned to one of three groups based on embryo cleavage results. In group I, one to two embryos per cycle showed irregular cleavage; group II, at least three embryos with abnormal cleavage; and in group III (the control group), all embryos cleaved normally. The number of embryos that cleaved from 1 to ≥ 3 cells or from 2 to ≥ 5 cells for each patient was recorded. Their prevalence and association with women's characteristics and pregnancy outcome were evaluated.

Results The prevalence of irregular cleavage was 15.6 % among 1772 ICSI embryos. In 101 cycles, 1–2 embryos per cycle showed irregular cleavage (group I). In 32 cycles, at least 3 embryos showed abnormal cleavage (group II). In 254 cycles, all embryos cleaved normally (group III). The average age of the women in group II was significantly lower

in comparison with groups I and III (32.5 ± 4.2 vs. 35.1 ± 4.9 and 35.5 ± 5.1 , respectively, $p < 0.02$). In comparison of groups I and II, the odds ratio for ≥ 3 embryos with irregular cleavage in women younger than 35 was 3.48 (95 % CI, 1.28 to 9.46). Embryos with irregular cleavage were transferred in 16 women. Three live births were achieved following the transfer of single blastocysts derived from embryos with irregular cleavage from two to five cells.

Conclusions Early embryos with irregular cleavage are significantly more prevalent in younger women. When these embryos develop to the blastocyst stage, they may have normal implantation potential, leading to the birth of healthy babies.

Keywords Embryo development · Implantation · Irregular cleavage · Pregnancy · Time-lapse

Introduction

Morphokinetic analyses of preimplantation human embryo development with time-lapse systems provide data that can be considered as markers of embryo quality and embryo implantation potential [1–4]. These analyses allow the embryologist to determine many morphokinetic parameters, such as the formation of pronuclei, the timing and symmetry of blastomere cleavage, blastocyst development, and embryo morphology. The correlation between embryo morphology and viability has been extensively documented and time-lapse imaging markedly increases the types of parameters available for assessing embryo quality. The phenomenon of a single cell division into more than two daughter cells has been reported in several research studies [5–7]. Morphokinetic annotations of cell divisions during the transition between the pronuclear and the eight-cell stage indicated that direct cleavage of embryos from one to three and from two to five blastomeres are

Capsule Prevalence of early embryos with irregular cleavage was significantly higher in younger women, and transfer of these embryos that reach the blastocyst stage may result in the birth of healthy babies.

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negatively associated with successful implantation [5, 6]. Recently, Wirka et al. [5] evaluated atypical dynamic embryo behaviors and found overall prevalence of 18 % abnormal cleavage among 639 embryos reviewed. These embryos showed significantly lower developmental potential than control group embryos. In light of the current literature, in this study, we have analyzed our results with time-lapse monitoring of early embryo development, focusing on patients' characteristics and pregnancy outcome when abnormal cleavage of embryos was documented.

Materials and methods

Study design

We performed a retrospective cohort study of all women who underwent assisted reproduction treatments in our unit at Kaplan Medical Center, Rehovot, Israel, from January 1, 2014 until January 31, 2015, irrespective of the women's age, cycle number, and reasons for treatment. Study approval was obtained from the Kaplan Medical Center Institutional Review Board.

Study inclusion criteria were as follows: patients undergoing intracytoplasmic sperm injection (ICSI) of fresh autologous oocytes, with at least one normal fertilization (two pronuclei) after injection of oocytes that showed first polar bodies. Only normally fertilized oocytes were evaluated. Patients with treatment cycles that included in vitro maturation or oocyte donation were excluded.

Each patient was assigned to one of three groups based on embryo cleavage results. In group I, one to two embryos per cycle showed irregular cleavage; group II, at least three embryos per cycle showed abnormal cleavage; and in group III (the control group), all embryos cleaved normally.

All embryos with irregular cleavage were scrutinized again to verify that they did divide in an irregular manner and to rule out the possibility that large un-nucleated fragments were mistaken for blastomeres.

Ovarian stimulation, ovum pick-up, ICSI, and culture conditions

Pituitary downregulation and controlled ovarian stimulation were carried out with a GnRH agonist or antagonist (Decapeptyl, Ferring, or Cetrotide, Merck Serono, respectively) followed by daily injections of gonadotropins. The daily doses were adjusted after 4–5 days according to follicle size and serum estradiol levels. Transvaginal oocyte retrieval was performed approximately 32–34 h after administration of hCG (Ovitrelle, Merck Serono Laboratories) or GnRH agonist or both.

Oocytes were cultured in a continuous single culture medium (CSC) with 10 % serum substitute supplement (Irvine Scientific, CA) or in Global Total for Fertilization (LifeGlobal, CT) at 5.5 % CO₂ and 37 °C before denudation with hyaluronidase (Sage CooperSurgical, CT). ICSI was performed in a medium containing HEPES buffer, and the injected oocytes were transferred into pre-equilibrated EmbryoSlide culture dishes (FertiliTech, Denmark) with 12 microwells. The oocytes were cultured individually in 25 µl of CSC or Global Total under a layer of oil in a time-lapse incubator (EmbryoScope, Unisense FertiliTech, Denmark) until day 3 or day 5 without media change or removal of the slides from the EmbryoScope.

Embryo grading, transfer, and implantation

Fertilization and embryo morphology were assessed based on the images acquired from the time-lapse system. Annotation of morphokinetics was performed on all embryos on culture days 1, 2, 3, and 5. Specifically, we focused on two types of abnormal cleavage observed on days 1–2: irregular first cleavage of the zygote, which resulted in more than two daughter cells and irregular second cleavage that formed five or more blastomeres instead of four.

Embryo transfers were performed on day 3 or 5. Serum levels of β -hCG were measured 14 days after oocyte retrieval. Clinical pregnancy was confirmed when an intrauterine gestational sac with a fetal heart beat was observed by ultrasound 3 weeks after a positive β -hCG result.

Statistical analysis

For women (the primary sampling unit) who underwent multiple cycles, only data on the first cycle was retained for the statistical analysis. This eliminated duplicates and provided a sample of independent subjects. For numeric variables, *t* tests were used following Levene's test for equality of variances. Dichotomies were tested using Fisher's exact test. Logistic regressions yielded odds ratios for three or more embryos with irregular cleavage. *P* values <0.05 were considered statistically significant.

Results

From January 1, 2014 until January 31, 2015, the Kaplan Medical Center IVF Unit performed a total of 387 ICSI treatment cycles in 253 women. One hundred seventy two (172) women (68 %) underwent one treatment cycle, 45 (18 %) underwent two cycles, and 36 (14 %) underwent 3–5 cycles.

Out of the 3001 oocytes retrieved, 2121 (71 %) oocytes were at metaphase II and 1772 embryos were obtained. The

average number of embryos transferred was 1.13 and 116 clinical pregnancies were achieved (30 % per cycle).

Of the 387 study cycles, in 133 (34 %) (groups I and II), a total of 276 embryos demonstrated irregular cleavage, with overall prevalence of 15.6 % among all ICSI embryos. Within the entire cohort of irregular cleavage (133 cycles), we identified 101 cycles in which 1–2 embryos per cycle showed irregular divisions (group I) as compared to 32 cycles in which at least 3 embryos per cycle had abnormal cleavage (group II). A total of 287 embryos were obtained in group II, out of which; 119 (41 %) demonstrated irregular cleavage. In the remaining 254 cycles performed during the study period, no irregular cleavage was observed (group III, control group).

The number of oocytes per cycle in group II (17.1 ± 8.7) was significantly higher than in groups I (9.8 ± 5.5) and III (6.4 ± 6.0), $p < 0.0001$. The average number of mature oocytes in this group was 14 ± 6.7 (mean percent $87 \% \pm 42 \%$) as compared to 8 ± 4.4 (mean percent $50 \% \pm 28 \%$) and 3.8 ± 3.1 (mean percent $56 \% \pm 31 \%$) in groups I and III, respectively, $p = 0.001$.

Characteristics of the study population are shown in Table 1. The average age of the patients was significantly lower in group II (32.5 ± 4.2) than both groups I (35.1 ± 4.9) and III (35.5 ± 5.1), $p < 0.02$.

No significant differences among the groups were found when comparing male factor infertility (40 %, 38 %, and 41 % for groups I, II, and III, respectively) and tubal factor infertility (21 %, 19 %, and 19 % for groups I, II, and III, respectively).

There was no correlation between ovulation induction protocols or type of culture media and the rates of irregular cleavage in the three groups.

In comparison of groups I and II, the odds ratio for ≥ 3 embryos with irregular cleavage in women younger than 35 was 3.48 (95 % CI, 1.28 to 9.46). The odds ratio increased

to 19.9 (95 % CI, 2.36 to 168.8) if more than 16 oocytes were retrieved.

It is noteworthy that in group II, out of 119 embryos with abnormal cleavage, 52 embryos (44 %) cleaved directly from 1 cell into 3 or more blastomeres, while the remaining 67 (56 %) underwent irregular second cleavages from 2 blastomeres to 5 or more. The rates of blastocyst formation in these 2 embryo populations were 35 % and 52 %, respectively.

In group I, 111 embryos demonstrated irregular cleavage. In 45 embryos (41 %), cleavage was from 1 to 3 cells and 31 % of them developed into blastocysts. Out of 66 embryos that cleaved from 2 to 5 blastomeres, 36 (55 %) became blastocysts.

Four women in group II who underwent two treatment cycles during our study period showed high rates of embryos with irregular cleavage in both cycles, as follows: first cycle and second cycle for each woman (4 of 8 and 6 of 13 embryos, 3 of 6 and 6 of 10 embryos, 3 of 3 and 5 of 10 embryos, 13 of 28 and 10 of 18 embryos). Only the first cycle of these women was included in the study.

Sixteen women (with ages ranging from 27 to 41) underwent transfers solely of embryos with irregular cleavage due to lack of normal cleavage embryos available for transfer. Three of these women, all aged 29, conceived following the transfer of a single blastocyst that developed from an embryo that cleaved from two to five cells. Three healthy babies have been born. No pregnancies were achieved from four embryos that cleaved directly from one to three cells.

There was no significant difference among the groups in the average number of embryos transferred (group I, 1.2 ± 0.7 ; group II, 1.1 ± 0.5 ; and group III, 1.1 ± 0.7), and there was no difference in pregnancy rate per transfer (group I, 37 %; group II, 40 %; and group III, 33 %).

Table 1 Patient demographics, cycle characteristics, and pregnancy outcome

	Group I: 1–2 embryos with irregular cleavage	Group II: ≥ 3 embryos with irregular cleavage	Group III: no irregular cleavage	<i>P</i> value
No. of cycles	101	32	254	
No. of women	92	26	135	
Age	35.1 ± 4.9	32.5 ± 4.2	35.5 ± 5.1	0.02*
Male factor infertility (%)	40	38	41	NS
Tubal factor infertility (%)	21	19	19	NS
Retrieved oocytes/cycle	9.8 ± 5.5	17.1 ± 8.7	7.7 ± 6.8	0.0001*
Metaphase II oocytes/cycle	8 ± 4.4	14 ± 6.7	3.8 ± 3.1	0.001
No. of embryos/transfer	1.2 ± 0.7	1.1 ± 0.5	1.1 ± 0.7	NS
Embryos with irregular cleavage	1.2 ± 0.4	4.5 ± 2.3	none	0.001
Pregnancies/embryo transfer (%)	37	40	33	

Note: Results are expressed as mean $\pm$ SD

NS not significant

*There was no difference between group I and group III, but both groups were significantly different from group II

Discussion

Spontaneous irregular cleavage of preimplantation human embryos from one to three cells or from two to five cells can be identified easily by continuous image acquisition in time-lapse incubators.

This report indicates that these types of irregular divisions are more prevalent in women of young ages. The findings also show that irregular second cleavage is compatible with successful implantation potential. The prevalence of abnormal cleavage in our study is comparable to the 18 % rate recently reported by Wirka et al. [5], but that study did not observe the relationship between cleavage rate and maternal age. This may be explained by the fact that Wirka et al. analyzed the prevalence of all atypical phenotypes among different age groups; however, they did not segregate the embryos with irregular cleavage for age analysis.

Although the number of oocytes recruited in the young women in our study was higher than the number in women of more advanced ages, the relative ratio of embryos with irregular cleavage as compared to embryos with normal cleavage was twice as high in the younger women. This shows a correlation between age and the number of irregularly dividing embryos. The odds ratio of 3.48 for irregular division in ≥ 3 embryos in women younger than 35 was significant ($p=0.02$). Moreover, when the number of aspirated oocytes was ≥ 16 , the odds ratio increased to 19.9 ($p=0.01$). This association is not fully understood and requires further study.

The possibility that low-quality sperm might contribute to the phenomenon of irregular cleavage was presented by Robio et al [6]. However, this does not explain the significantly lower rate of irregular cleavage in women in group I, despite similar rates of male factor infertility.

We observed that a high rate of irregular cleavage was repeated in consecutive cycles of several women, indicating that modifications in individual protocols of ovulation induction were not strongly associated with the mitotic behavior of the zygotes. Most aspirated oocytes are at the stage of metaphase II and develop two pronuclei after ICSI; yet, we surmise that in some, the cascade responsible for the activation of mitosis is disturbed. It ought to be mentioned that bovine zygotes that underwent direct division from one to three cells had developmental ability comparable to embryos with normal division, but with a high rate of chromosomal abnormalities [8].

The molecular mechanisms that underlie abnormal cleavage are not clear. Yet, it seems reasonable to assume that mitotic errors may play an important role in this phenomenon although all the embryos we analyzed developed from 2PN zygotes. It has already been shown that three-pronuclear human zygotes display a high rate of first cleavage into three blastomeres [9]. This suggests abnormal distribution of chromosomes during first mitosis. Chavez et al. [10] observed that

the generation of partial chromosomal gains and losses were restricted primarily to embryos with mitotic errors. During the first mitotic divisions in human preimplantation development, improper segregation of chromosomes might occur, causing high rates of aneuploidy and mosaicism [11]. Several studies showed that a majority of preimplantation embryos are diploid-aneuploid mosaic [12–14]. Chow et al [14] applied array comparative genomic hybridization to analyze high percentage of blastomeres from good quality day 3 embryos of young patients (with no known indication for PGD). These embryos showed a high degree of mosaicism and less than 20 % euploidy. It is likely that mosaic embryos with a low number of abnormal blastomeres have self-correction mechanisms that enable normal implantation [13, 14]. It has been shown that abnormal cells can be forced away from the embryonic inner cell mass leading to a viable euploid embryo [11]. There may be preferential growth of euploid blastomeres and/or reduced division of aneuploid cells. It has also been suggested that the development of cell cycling genes after embryonic genome activation at the eight-cell stage reduces the proportion of aneuploid cells [14].

Because we have a high rate of single-embryo transfers in our unit, we have been able to identify the implantation potential and live birth outcomes of embryos with irregular cleavage in our research groups. Based on our results, we hypothesized that in cases with second irregular cleavage, only one blastomere undergoes abnormal cleavage, whereas the other one cleaves normally to yield a proper chromosomal balance, which may give rise to a viable embryo. On the other hand, in abnormal first cleavage, the implantation potential is limited because the zygote has no reserve cell that could cleave normally. Our assumption is based on the fact that we were able to achieve pregnancies and live births only from embryos with abnormal second cell divisions. No pregnancies were achieved from the transfer of four embryos with abnormal first cleavage. These results are supported by Wirka et al. [5] who noted in their study that one embryo with abnormal second cleavage was transferred on day 5 and implanted successfully, whereas no embryos with abnormal first cleavage implanted. However, Stecher et al. [15] described a successful delivery of two healthy dizygotic twins following transfer of two blastocysts, one of which developed from an embryo that cleaved directly from one to three cells. Thus, even when embryos demonstrate unusual morphokinetic patterns, different growth rates of normal and abnormal cells limit adverse events in early embryo development and finally lead to a chromosomally normal fetus [14, 15].

One limitation of our study is that it is a retrospective analysis. Furthermore, since we only transfer embryos with irregular cleavage when no normally cleaving embryos are available, our sample size is small. Sixteen embryos with irregular cleavage were transferred, 4 from 1 to 3 cells (3 blastocysts) and 12 from 2 to 5 cells (6 blastocysts). This sample size is too

small to reach a sound conclusion and a larger sample size is needed to determine their true implantation potential.

In conclusion, we feel that it is appropriate to suggest that embryos with irregular cleavage that reach the blastocyst stage may be transferred or cryopreserved for future transfers.

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Conflict of interest The authors declare that they have no competing interests.

Ethics approval Study approval was obtained from the Kaplan Medical Center Institutional Review Board.

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