Blastomere Cytoplasmic Granularity is Unrelated to Developmental Potential of Day 3 Human Embryos

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Purpose: To examine whether the increase in cytoplasmic granularity observed in some human embryos on day 3 of development is of any predictive value as to embryo developmental potential.

Methods: Retrospective study comparing outcomes of treatment attempts in three groups of patients after day 3 embryo transfer. Attempts in which only embryos with clear cytoplasm were transferred constitute Group I, those in which only embryos with granulated cytoplasm were transferred constitute Group II, and Group III consists of cases with mixed transfer combining both types of embryos. Each group was further divided according to the female age.

Results: Clinical pregnancy rates in Groups I (314 attempts), II (173 attempts), and III (323 attempts) were 33.8, 36.4, and 31.3%, respectively. Implantations rates for Groups I–III were 17.0, 17.3, and 14.8%, respectively. No significant differences between groups concerning these and other values, including the number of oocytes and of metaphase II oocytes recovered, fertilization and cleavage rates were found. The proportion of good-morphology embryos was also similar between the different groups (74.3, 72.7, and 70.2% respectively). The representation of women of advanced age (>36 years) was also similar in each group, and intergroup differences remained insignificant were only younger or only older women were taken into account.

Conclusion(s): These data show that the appearance of cytoplasmic granulation in blastomeres of day 3 human embryos is of no prognostic value as to embryo quality and appears to be unrelated to the female age.

KEY WORDS: Blastomere cytoplasmic granularity; developmental potential; human cleaving embryo.

INTRODUCTION

The cytoplasm of human cleaving embryos is usually considered normal when it is pale, and clear or finely granular in appearance (1), although the definitive norm has yet to be established. In fact, some authors have reported an increase in cytoplasmic granularity and the appearance of tiny pits in the cytoplasm of

blastomeres of day 3 human embryos (2,3). It was suggested that this phenomenon might represent an early marker of the blastomeres cytoplasmic activity at the outset of compaction and that it is associated with embryos most likely to proceed on to the morula and blastocyst stages (2,3). In fact, a quantitative electron microscopic analysis of human preimplantation embryos has shown that an extensive repartition of intracellular membranes amongst different types of organelles is the earliest detectable morphological marker of the major activation of embryonic genome at the 8-cell stage (4), and these changes may well underlie the cytoplasmic granularity observed in living human embryos.

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Fig. 1. Day 3 embryo with clear cytoplasm (A) and day 3 embryo exhibiting cytoplasmic granularity (B).

Preliminary observation of an association between the occurrence of cytoplasmic granularity at the 8-cell stage and embryo development to the blastocyst stage was made with a group of younger patients being transferred on day 5 (3). The impact of this variable became less pronounced and lost statistical significance when the same authors extended their study to a larger group of patients including those of >39 years of age (3).

This study was undertaken to determine whether the occurrence of cytoplasmic granularity in day 3 human embryos has some predictive value as to embryo quality in a large, unselected patient population, and in age-selected subpopulations of younger (≤ 36 years) and older (> 36 years) women.

MATERIALS AND METHODS

This retrospective study⁴ included 810 consecutive ICSI cycles using ejaculate sperm performed at the Centre for Reproductive Medicine at the European Hospital of Roma, Italy between January 2000 and December 2001. Controlled ovarian stimulation was performed as described previously (5) with the use of recombinant human FSH (Puregon, Organon, Oss; Netherlands or Gonal-F; Serono, Rome, Italy) after pituitary suppression with gonadotrophin-releasing hormone agonist (GnRHa; busserelin acetate; Suprefact; Hoechst, Marion Roussel, Milan, Italy) started in late luteal phase of the previous cycle and human chorionic gonadotrophin (HCG; Profasi, Serono). Oocytes and ejaculated semen preparation as well

as ICSI procedure have been extensively described elsewhere (6). Fertilization was considered normal when two clearly distinct pronuclei (PN) and two polar bodies were present between 12 and 16 h after ICSI procedure. Only normally fertilized oocytes (zygotes) were considered further for eventual embryo transfer or cryopreservation. All zygotes were cultured in IVF medium (Vitrolife, Gothenburg, Sweden) up to day 3 after ICSI. Cleaving embryos were evaluated on days 2 and 3 with the use of a cumulative embryo classification scheme as described previously (7). With respect to this scheme, an embryo was considered excellent when it scored 0–1, good when it scored 2–3, poor when it scored ≥ 4 . The best-scoring embryos were selected for transfer and the remaining excellent and good quality embryos were cryopreserved. The luteal phase was supported by daily means of natural progesterone in oil (Prontogest 100 mg; Amsa, Barberino del Mugello, Italy). Pregnancy was confirmed by a serial rise in serum HCG concentration on two consecutive occasions, 12 days after embryo replacement. Clinical pregnancy was determined by ultrasound demonstration of cardiac activity at 7 weeks.

In addition to the above characteristics, blastomeres cytoplasmic appearance was also recorded by the same observer on day 3 in all embryos included in this study. However, this feature was not considered for embryo selection for transfer. The embryos were classified as “clear” when the blastomeres cytoplasm was pale, and clear or finely granular and as “granular” when the majority of blastomeres displayed a uniform granulated cytoplasm and when tiny pits were present (Fig. 1). All these attempts were subsequently analyzed in a retrospective manner for the relationship between blastomere cytoplasmic granularity and pregnancy and implantation rates. For the purpose of this study attempts in which only embryos with clear

⁴ Institutional Review Board (IRB) approval was obtained for this study.

Table I. Number of Oocytes and Fertilization Outcomes by Group

Group	No. of oocytes (mean $\pm$ SD)		2 PN zygotes (%) ^a	No. (%) of embryos ^b			
	Total	Metaphase II		Total	Excellent	Good	Poor
I (<i>n</i> = 314)	10.8 $\pm$ 7.1	9.5 $\pm$ 6.0	1927 (64.5)	1872 (97.1)	663 (35.4)	729 (38.9)	479 (25.6)
II (<i>n</i> = 173)	12.1 $\pm$ 6.1	10.2 $\pm$ 5.5	1237 (69.9)	1203 (97.3)	394 (32.7)	483 (40.0)	320 (26.6)
III (<i>n</i> = 323)	12.1 $\pm$ 7.0	10.7 $\pm$ 6.0	2278 (66.2)	2246 (98.6)	677 (30.1)	901 (40.1)	642 (28.6)

^a Percentages are calculated from the number of metaphase II oocytes.

^b Percentages are calculated from the number of 2PN zygotes.

cytoplasm were transferred from Group I, those in which only embryos with granulated cytoplasm were transferred constitute Group II, and Group III consists of cases with mixed transfer combining both types of embryos. Moreover, each group was divided according to the female age. We choose a cut off of 36 years because as observed by others (8) and according to the clinical results of our centre we observe an important decline of the pregnancy and implantation rates after this age.

The differences in the female age, number of CCOCs, metaphase II oocytes recovered and the number of embryo transfer procedures performed were compared with the use of paired Student's *t*-test. Differences in the fertilization, cleavage, clinical pregnancy, and implantation rates between the groups were evaluated by χ^2 test. A *p* value <0.05 was considered significant.

RESULTS

The mean age ($\pm$ SD) of female patients did not differ between Groups I, II, and III and was 35 ± 4.4 , 35 ± 4.1 , and 35 ± 4.2 years, respectively. The total number of oocytes recovered, the number of metaphase II oocytes, the total number of zygotes as well as the number and percentage of all embryos obtained and of embryos showing different morphology grades calculated by each of the Groups I–III are shown in Table I. No differences in any of these parameters between groups were found.

Clinical pregnancy and implantation rates by group are presented in Table II. Here again no differences were detected between Groups I–III. The number

of abortions in Groups I–III was 10, 3, and 6, respectively, resulting in the respective ongoing pregnancy rates of 31, 34.7, and 29.0% (differences not significant).

The percentages of treatment attempts included to Groups I–III were not different when only attempts with female age of >36 years or only those with female age of ≤ 36 years were taken into account. Out of 348 cases with female age of >36 years, 127 (40.5%) were classified as Group I, 72 (41.6%) as Group II, and 149 (46.1%) as Group III. The corresponding data for Groups I–III when only cases with female age of ≤ 36 years (*n* = 462) were taken account were 187 (59.5%), 101 (58.4%), and 174 (53.9%), respectively. No significant differences between these subgroups concerning number of oocytes and of metaphase II oocytes recovered, fertilization and cleavage rates were found. Clinical pregnancy (26.7, 27.8, and 20.8%, respectively) and implantation rates (13.5, 11.3, 10.7%) in Groups I, II, and III with female age of >36 years were also not significantly different. The same comparisons also failed to give statistically significant differences between Groups I–III when only cases with female age of ≤ 36 years were taken into account.

DISCUSSION

This study failed to demonstrate any relationship between the occurrence of cytoplasmic granularity in blastomeres of day 3 human embryos and the embryo developmental potential. Moreover, there was no relationship between blastomere cytoplasmic

Table II. Clinical Pregnancy and Implantation Rates by Group

Group	No. of transfers	No. of embryos transferred	Clinical pregnancy rate	Implantation rate
I	314	2.8 $\pm$ 1.0	106/314 (33.8%)	150/879 (17.0%)
II	173	2.8 $\pm$ 1.0	63/173 (36.4%)	85/490 (17.3%)
III	323	2.8 $\pm$ 0.9	101/323 (31.3%)	135/913 (14.8%)

granularity, on the one hand, and the age of the female patient, the number of metaphase II oocytes recovered, fertilization rate or blastomere regularity and fragmentation on the other hand. The data, obtained with a relatively high number of embryos (810 treatment attempts and 2282 transferred embryos) are in agreement with a preliminary report based on a much lower number of specimens (93 treatment attempts and 316 transferred embryos) in which no relationship was found between the number of embryos showing blastomere cytoplasmic granularity (pitting) and the clinical pregnancy rate (3).

However, the authors of the latter report refer to an unpublished series of cases in which only younger patients were included and in which blastomere cytoplasmic granularity was associated with embryos most likely to proceed on to the morula/blastocyst stage (3). We thus evaluated the relationship between blastomere cytoplasmic granularity separately for treatment attempts with lower (≤ 36 years) and higher (> 36 years) female age. Notwithstanding, the same results, confirming the absence of detectable relationship between blastomere cytoplasmic granularity and any of the outcome measures used in this study, were obtained in both age groups.

These results strongly suggest that the early morphological signs of embryonic genome expression, detectable in human 8-cell embryos by quantitative transmission electron microscopy (4), are not reflected by any change in the texture of blastomere cytoplasm which could be detected in living embryos observed in a light microscope. In fact, combined examination of blastomere ultrastructure and ^3H -uridine incorporation has shown that many human embryos possess at the same time some blastomeres with morphological signs of embryonic genome expression and with active RNA synthesis and other blastomeres in which these signs of early genomic activity are lacking (4). In contrast, we never observed

embryos having some blastomeres with granulated cytoplasm and others with clear cytoplasm in this study. This also suggests that blastomere cytoplasmic granularity in day 3 human embryos is not related to embryonic genome activity and probably is caused by factors that do not influence embryo viability.

In conclusion, the texture of blastomere cytoplasm in day 3 human embryos has no predictive value and should not be used as an additional criterion in the selection of embryos for transfer or in the determination of the number of embryos to be transferred.

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