Article

Impact of the assessment of early cleavage in a single embryo transfer policy

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Abstract

The policy of single embryo transfer (SET) adopted for women <36 years old since 1 July 2003, strongly calls for improvement of embryo selection. A total of 196 cycles in which SET was performed were randomly allocated to two groups. In the first group, early cleavage was assessed (ECA) 25 h after insemination. The embryo with the best score that cleaved early, if present, was selected for transfer. In the second group, early cleavage was not assessed (ECNA) and embryo selection was based solely on the embryo score. Ninety-eight cycles were allocated in the ECA and ECNA group respectively. Early cleavage occurred in 64% of cycles and 32.2% of embryos. Patient population and embryo morphology were similar between the two groups, and similar delivery rates were observed (27.6 versus 24.5% respectively in the ECA and ECNA groups). The assessment of early cleavage as additional parameter did not improve the delivery rate in the single embryo transfer policy.

Keywords: *early cleavage, elective single embryo transfer, IVF*

Introduction

Since 1 July 2003, the Belgian Social Security System has imposed a new transfer policy to reduce the percentage of twin pregnancies that are known to increase the risk of obstetrical and fetal complications for the mother and the newborn (Elster, 2000; Olivennes, 2000; Land and Evers, 2003). The current prospective study was undertaken to verify if assessment of early cleavage has additional value to the embryo selection procedure used, based solely on embryo score, and if it could improve clinical pregnancy rate in a single embryo transfer (SET) policy. The objective of this policy is also to reduce the medical costs of twin pregnancies (Elster, 2000; Kinzler *et al*., 2000). In practice, women under the age of 36 have one embryo replaced regardless of the embryo quality in first reimbursed cycle, and one embryo replaced in second reimbursed cycle if it is of top quality. Consequently, for this group of patients, there was a unique opportunity to examine the correlation between embryo characteristics and implantation rate. Several previous studies have suggested that the assessment of early cleavage of zygotes occurring by 25–27 h after insemination could be used to predict the implantation potential of embryos, (Edwards *et al*., 1984; Shoukir *et al*., 1997; Sakkas *et al*., 1998a, 2001; Bos-Mikich *et al*., 2001; Lundin *et al*., 2001; Petersen *et al*., 2001; Fenwick *et al*., 2002; Isiklar *et al*., 2002; Brezinova *et al*., 2003, 2004; Salumets *et al*., 2003; Ciray *et al*., 2004; Van Montfoort *et al*., 2004), but these studies were retrospective and in most of them more than one embryo was replaced. Two studies performed single embryo transfers (Salumets *et al*., 2003; Van Montfoort *et al*., 2004). One group showed that the early cleavage assessment could improve the embryo selection by a prospective randomized trial in a multiple embryo transfer policy (Sakkas *et al*., 2001). Finally, one group observed the same implantation rate when early cleavage was randomly checked and not checked (29.8 versus 27.5%) in a multiple transfer policy (Ciray *et al*., 2005). Embryos are usually selected following the scoring system proposed by Puissant *et al*. (1987), in which the transfer priority is given to embryos with the higher cleavage rate, lower fragmentation rate and regular blastomere shape on day 2.

Materials and methods

Inclusion criteria

For the new transfer policy introduced by the Belgian Social Security since 1 July 2003, it is necessary to transfer one embryo in patients younger than 36 years, performing their first reimbursed cycle, while it is permitted to select a second embryo in the second reimbursed cycle, only in cases of low quality of the best scoring embryo: all couples undergoing their first or second reimbursed IVF or intracytoplasmic sperm injection (ICSI) cycle with a minimum of two fertilized oocytes and that received one embryo were included in the study.

Randomization

Patients were randomized in two groups on the basis of their inclusion in a randomization list with permuted blocks. A total of 93 patients who performed 98 IVF or ICSI cycles were allocated to the group with early cleavage assessment (ECA group), while 94 patients who underwent 98 IVF or ICSI cycles were allocated to the group with no early cleavage assessment (ECNA group). The randomization was performed in the IVF laboratory on the day of oocyte retrieval. Four cycles were excluded from the analysis in the ECA list: one for fertilization failure and three because the patient performing the second reimbursed cycle received two embryos.

Stimulation protocol and oocyte recovery

Ovarian stimulation was performed using gonadotrophin-releasing hormone analogue (buserelin acetate: Suprefact spray; Hoechst Inc. Frankfurt, Germany), gonadotrophins (Puregon; Organon Inc. Oss, The Netherlands; Gonal F; Serono Inc., Rome, Italy) and human chorionic gonadotrophin (HCG, Pregnyl, Organon Inc). Oocyte retrieval was performed through vaginal puncture under ultrasound guidance 36 h after the injection of 10,000 IU of HCG.

Culture condition system

Embryo culture was performed in a sequential 'in-house' medium. Culture medium composition and culture conditions have been previously described (Emiliani *et al.*, 2003).

Early cleavage assessment and embryo transfer policy

In the ECA group, early cleavage was assessed 25 h after insemination, performed either by IVF or ICSI. The embryo that cleaved early, if present, and with the best score on day 2 (Puissant *et al.*, 1987) was selected for transfer. It means that if two or more embryos were observed with the same score, the one that cleaved earliest (if present) was selected, so that the early cleavage was considered only as an additional parameter to classical embryo score. A score of four points was given for a 2-cell embryo with regular blastomeres and no anucleate fragments, three points for a 2-cell embryo with uneven blastomeres, or fragments ≤1/3 of the embryonic surface, and scores two or one for a 2-cell embryo with uneven blastomeres and fragmentation >1/3 of the embryonic surface. Two more points were added if the embryo had reached the 4-cell stage. In the control group (ECNA group), the early cleavage was not assessed and the embryo selection was performed only on the basis of the embryo score. Embryo replacement was performed in both groups on day 2. Clinical pregnancy rate was defined as the number of pregnancies in which a gestational sac was observed on ultrasound examination 28 days after the oocyte retrieval.

Statistical analysis

The data were analysed with the statistical Package for Social Science SPSS version 7.5 for Windows 98, under the licenses obtained by the fertility clinic from SPSS Inc. All data were checked for their normal distribution by submission to the Kolmogrov–Smirnov test and, if significant, non-parametric statistical analysis was applied.

For other cases data were analysed using Student's *t*-test. The chi-squared test was used when necessary with Yate's correction. $P < 0.05$ was considered as statistically significant.

Results

The two groups were similar for the mean age of the patients, the mean number of collected oocytes, the mean number of previous attempts and the proportion of IVF and ICSI cycles (**Table 1**). Sixty-four percent (60/94) of cycles had at least one early-cleaved embryo (61% in IVF and 65% in ICSI cycles: χ^2 = not significant). Among these cycles, 32.2% (210/653) of the embryos cleaved 25 h post-insemination and 38 of them were replaced. No differences were observed between the two groups in fertilization rate, day 2 cleavage rate and mean score of total embryos (**Table 2**). Furthermore, the mean score of replaced embryos was not different even if the day-2 cleavage rate of replaced embryos was slightly higher in the ECA group (**Table 3**).

The delivery rates in the ECA and ECNA groups were not different (27.6 versus 24.5% respectively). A total of 38 pregnancies were obtained in the ECA group: one extrauterine, eight biochemical, three miscarriages and 26 deliveries. In the ECNA group, 35 pregnancies were obtained, five biochemical, five miscarriages, one extrauterine and 24 deliveries.

When, in the ECA group, embryos coming from 60 cycles in which at least one embryo cleaved early (EC+) were compared with 34 cycles in which no early-cleaving embryos were observed (EC−), it was noticed that even if the two groups were similar in the patient population, the mean number of cells and the score of all embryos were significantly higher in the EC+ cycles (Mann–Whitney *u*-test: $P = 0.05$ and $P < 0.01$ respectively) (**Table 3**). On the other hand, the difference in embryo score disappeared when only replaced embryos were compared (**Table 3**). When, in the EC+ group, embryos that cleaved early and did not cleave early were compared, a significant difference was observed in the mean number of cells 46 h post-fertilization (3.88 ± 0.77 versus 3.55 ± 0.98 respectively) (Mann–Whitney *u*-test: $P < 0.01$) and in the mean embryo score (4.10 ± 1.20 versus 3.68 ± 1.28 respectively) (Mann–Whitney *u*-test: $P = 0.01$) (**Table 3**). Furthermore, a significant and positive correlation

Table 1. Patient population and clinical results in inspected and not inspected cycles.

Parameter	*ECA*	*ECNA*
No. of patients	90	94
No. of cycles	94	98
Mean age ± SD	30.31 ± 3.31	30.14 ± 3.31
Mean no. of oocytes ± SD	11.33 ± 5.11	10.88 ± 5.30
Mean no. of attempts ± SD	2.22 ± 2.10	2.00 ± 1.33
IVF (*n*)	36	34
ICSI (*n*)	58	64
Delivery date (%)	26/94 (27.6)	24/98 (24.5)

ECA = early cleavage assessed; ECNA = early cleavage not assessed.

was observed between embryo score and early cleavage (r = 0.165 and 0.178 respectively; Pearson: $P < 0.01$).

Within the 60 EC+ cycles 17 deliveries (28.3%) were obtained, while nine deliveries (26.5%) were obtained within the 34 EC– cycles. In 38 cycles, the embryo selected for transfer (with the best score) had also presented an early cleavage, resulting in nine deliveries (23.4%). In 22 cycles, the embryo selected for transfer had not presented an early cleavage resulting in eight deliveries (36.4%). While the same proportion of embryos was frozen in ECA and ECNA group (26.9 versus 30.7%), a significantly higher percentage of embryos was frozen in the EC+ cycles than in EC– cycles (30.6 versus 19%, respectively) (χ^2: $P < 0.01$).

Table 2. Day 2 embryo morphology in inspected and not inspected cycles.

Parameter	*ECA all embryos*	*ECNA all embryos*
No. of cells (mean ± SD)	3.63 ± 0.98	3.57 ± 1.06
Score (mean ± SD)	3.71 ± 1.26	3.78 ± 1.23
	Replaced embryos	*Replaced embryos*
Mean no. of cells ± SD	4.00 ± 0.60[a]	3.83 ± 0.61[a]
Mean score ± SD	4.80 ± 0.92	4.93 ± 0.94

ECA = early cleavage assessed; ECNA = early cleavage not assessed.
[a]Mann-Whitney *u*-test: $P = 0.03$.

Table 3. Comparison of parameters within the ECA group between cycles with early-cleaved embryos (EC+) and cycles with no early-cleaved embryos (EC–).

Parameter	*EC+*	*EC–*
No. of cycles	60	34
Mean no. of oocytes ± SD	12.03 ± 5.30	10.09 ± 4.40
Mean age ± SD	30.13 ± 3.40	30.88 ± 3.10
Mean no. of attempts ± SD	2.15 ± 1.90	2.35 ± 2.45
Fertilization rate%	65.5	60.30
Delivery rate (%)	17/60 (28.3)	9/34 (26.5)
All embryos		
Mean no. of cells ± SD	3.69 ± 0.91[a]	3.50 ± 1.10[a]
Mean score ± SD	3.85 ± 1.26[b]	3.40 ± 1.22[b]
Replaced embryos		
Mean no. of cells ± SD	4.05 ± 0.43	3.91 ± 0.83
Mean score ± SD	4.90 ± 0.90	4.59 ± 0.96
In EC+ cycles only	*Embryos that cleaved early*	*Embryos that did not cleave early*
Mean no. of cells ± SD	3.88 ± 0.77[b]	3.55 ± 0.98[b]
Mean score ± SD	4.10 ± 1.20[c]	3.68 ± 1.28[c]

EC+ = cycles with early-cleaved embryos; EC– = cycles with no early-cleaved embryos.
[a,b,c]Mann–Whitney *u*-test: $P < 0.05$, $P < 0.01$; $P = 0.01$ respectively.

Discussion

This study tested whether the assessment of embryo early cleavage 25 h after fertilization, a procedure that requires a further manipulation of embryos, is useful to improve embryo selection in a single embryo transfer policy, in a selected patient population, or is redundant. The practice of SET allowed precise analysis of embryo features and clinical results. The results of the randomized study showed similar clinical delivery rates for both groups with or without assessment of early cleavage as an additive parameter for embryo selection (27.6 versus 24.5% respectively).

The positive correlation observed between the embryo score and the presence of an early cleavage (Pearson: $P < 0.01$) indicates that the two variables are dependent. Furthermore, in a SET policy a single top quality embryo was selected for transfer, and this procedure eliminated differences in the score of replaced embryos between the two groups. The two factors together could explain why the assessment of early cleavage to select the best embryo was redundant and did not increase the delivery rate in the checked group.

Furthermore, the uselessness of early cleavage inspection in multiple transfer policy and in an unselected patient population was shown by Ciray *et al.* (2004), in a study in which implantation rates of 27.5 and 29.8% were respectively observed when not inspected, and inspected cycles were prospectively randomized. Thus, these data were confirmed, but with a different patient population and embryo transfer policy.

Currently many IVF programmes apply several embryo grading systems for embryo selection (Puissant *et al.*, 1987; Hill *et al.*, 1989; Scott *et al.*, 1991; Steer *et al.*, 1992) and several previous studies have shown that higher implantation rates were obtained when EC embryos were replaced (Shoukir *et al.*, 1997; Sakkas *et al.*, 1998a, 2001; Bos-Mikich *et al.*, 2001; Lundin *et al.*, 2001; Petersen *et al.*, 2001; Fenwick *et al.*, 2002; Brezinova *et al.*, 2003, 2004; Salumets *et al.*, 2003; Cyray *et al.*, 2004, 2005; Van Montfoort *et al.*, 2004). A positive correlation between embryo score, embryo viability and early cleavage was also observed by several authors and confirmed in the present study (Lundin *et al.* 2001; Salumets *et al.*, 2003; Cyray *et al.* 2004, 2005; Van Montfoort *et al.*, 2004) and many factors were analysed to try to explain this correlation. Included is the difference in the time necessary for spermatozoa to penetrate the zona pellucida and differences in oocyte maturity (Shoukir *et al.*, 1997; Sakkas *et al.*, 1998a), paternal factors (Sathananthan *et al.* 1991; Parinaud *et al.* 1993; Van Steirtheghem *et al.*, 1993; Janny and Ménézo, 1994; Nagy *et al.*, 1994; Payne *et al.*, 1994; Tournaye *et al.*, 1995; Palermo *et al.* 1997; Sakkas *et al.*, 1998a,b; Sathanantan, 1998; Obasaju *et al.*, 1999; Larson *et al.* 2000), chromosomal status of embryo (Barrenäs *et al.*, 2000; Baltaci *et al.*, 2006), intrinsic factors within the oocyte (Goldbard and Warner, 1982; Brownell and Warner, 1988; Stroynowski, 1990; Jurisicova *et al.*, 1996; Lundin *et al.*, 2001), duration of first oocyte cleavage that seems to be related to the amount and/or quality of RNA or protein stored in the oocyte and consequently to the embryo developmental potential (Grisart *et al.*, 1994).

The selection of a critical time point for checking embryo early cleavage is essential. The assessment of early cleavage between 25 and 29 h post-fertilization was previously described (Shoukir *et al.*, 1997; Sakkas *et al.*, 1998a, 2001; Bos-Mikich *et al.*, 2001; Lundin *et al.*, 2001; Petersen *et al.*, 2001; Fenwick *et al.*, 2002; Brezinova *et al.*, 2003, 2004; Salumets *et al.*, 2003; Cyray *et al.*, 2004; Van Montfoort *et al.*, 2004). The timing of first cell division in humans has been reported to be between 20 and 22 h (Balakier *et al.*, 1993) and 25 h (Capmany *et al.*, 1996). A surprisingly higher percentage of cycles in which one cleaved embryo was observed in 25 h post-insemination was observed in this study (64% of cycles), in comparison with others in which a proportion of early-cleavage positive cycles was included between 9.5 and 59% at the same time-point for checking (Shoukir *et al.*, 1997; Sakkas *et al.*, 1998a, 2001; Bos-Mikich *et al.*, 2001; Lundin *et al.*, 2001; Petersen *et al.*, 2001; Fenwick *et al.*, 2002; Brezinova *et al.*, 2003, 2004; Salumets *et al.*, 2003; Cyray *et al.*, 2004; Van Montfoort *et al.*, 2004). This difference could be explained by differences in embryo culture conditions existing between IVF laboratories (Gardner *et al.*, 2005), in sperm characteristics (Sathanantan *et al.*, 1991, 1998; Palermo *et al.*, 1997) and women's age (Lundin *et al.*, 2001; Ciray *et al.*, 2004), all factors that can influence embryo cleavage rate. Further factors predictive for embryo implantation were reviewed by Borini *et al.* (2005), and between them the pronuclear morphology, follicular vascularization, embryo morphology, blastocyst development. What one should try to establish is which factors are interdependent, to eliminate redundant embryo parameter evaluations.

Finally, even if no difference in pregnancy rate was observed between EC+ and EC− cycles, the higher proportion of frozen embryos in EC+ could have a positive cumulative effect of frozen/thawed cycles in the overall pregnancy rate of this group (Gabrielsen *et al.*, 2005).

Early cleavage is now used in some clinics to select embryos for transfer, but only to make a distinction between embryos of identical embryo score (Sakkas *et al.*, 1998; Salumets *et al.*, 2003). In this study, this parameter did not have priority on the embryo score on day 2, and it was considered only as an additional parameter. To date, no other studies have been published in which embryo selection for transfer was performed only on the basis of early cleavage, regardless of embryo score.

In conclusion, even if the early cleaved embryos showed a higher developmental potential in comparison with late cleaved embryos, the correlation between early cleavage and embryo score and the selection of a top quality embryo makes redundant the use of this parameter as additive in a single embryo transfer policy.

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