



ORIGINAL ARTICLE

Does early cleavage correlate with chromosome constitution in human preimplantation embryos?



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KEYWORDS

Early cleavage (EC);
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Abstract

Introduction: The purpose of this study is to analyze the correlation between the occurrence of early cleavage and chromosome constitution of embryos from preimplantation genetic screening (PGS) and preimplantation genetic diagnosis (PGD).

Material and methods: A total of 595 embryos from 96 cycles were included in this retrospective study.

Results: When clinical pregnancy rates per transfer were compared, statistically significant differences were observed between patients that had at least one early cleavage (ECpos) embryo and patients no EC (ECneg) (35.7% vs. 6.7%; $p < .001$). Statistically significant differences were found between EC, No PN and 2PN embryos at 26 h, good embryo quality at day 2 (71.4%, 55.6% and 40.7%, respectively; $p < .05$) and in blastocyst rate (43.3% EC vs. no early cleavage embryos (NEC) 27.1%; $p < .001$) in PGS cycles. These differences were not found in the PGD group. Early-cleaved embryos exhibited less chromosome abnormalities than No PN and 2PN group in PGS (61%, 69.4% and 77.2%; $p < .05$) and differences in PGD group (69.8%, 76.2% and 66.7%; $p < .05$).
Discussion: Early cleavage has shown to correlate with embryo quality, with the capacity to develop up to blastocyst stage, as well as with euploid chromosome constitution.

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PALABRAS CLAVE

División temprana (EC);
Calidad embrionaria;
Cribado de aneuploidías (PGS);
Diagnóstico genético preimplantacional (PGD)

Existe una correlación entre la división temprana de los embriones y la dotación cromosómica de los embriones preimplantacionales humanos?

Resumen

Introducción: El objetivo de este estudio es analizar la relación que existe entre la división temprana (EC) y la dotación cromosómica de los embriones del Programa de Diagnóstico Genético Preimplantacional (PGD) y Cribado o Screening de Aneuploidías (PGS).

Material Y Métodos: Se han incluido un total de 595 embriones provenientes de 96 ciclos. Estudio retrospectivo.

Resultados: Se han observado diferencias estadísticamente significativas en la tasa de embarazo entre las pacientes con algún embrión EC (ECpos) y ningún embrión EC (ECneg) (35.7% vs. 6.7%; $p < 0.001$). Se han encontrado diferencias estadísticamente significativas entre el estadio del embrión a las 26 h: EC, No PN y 2PN, y la buena calidad embrionaria en día 2 (71.4%, 55.6% y 40.7%; $p < 0.05$); así como también en la tasa de formación de blastocisto (43.3% EC vs. No early cleavage (NEC) 27.1%; $p < 0.001$) en los ciclos PGS. Estas diferencias no se ha encontrado en el grupo de PGD. Los embriones EC presentan menos anomalías cromosómicas que los No PN y los 2PN en el grupo de PGS (61%, 69.4% y 77.2%; $p < 0.05$) y también existen diferencias en el grupo de PGD (69.8%, 76.2% y 66.7%; $p < 0.05$).

Discusión: La división temprana del embrión está relacionada con la calidad embrionaria, la capacidad de desarrollo hasta blastocisto y con dotación cromosómica euploide.

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Introduction

When performing in vitro fertilization (IVF), the selection of the embryo with the best prognosis and highest implantation potential is essential in order to reduce the number of embryos to be transferred, and therefore the risk of multiple pregnancy, which can lead to maternal and perinatal complications without reducing the pregnancy rate (Bergh et al., 1999; Devroey et al., 2009). The most widely used criteria for the evaluation of embryo quality is the assessment of pronuclear morphology at 18–20 h after insemination (day 1), embryo morphology and cleavage stage at 44–47 h and 67–71 h after insemination (day 2 and day 3) and/or blastocyst morphology (day 5 to day 6).

Another embryo quality indicator is the assessment of early cleavage (EC) at 25–27 h after insemination (Edwards et al., 1984). It has been suggested that the assessment of early cleavage is less subjective and more reliable, fast and simple to routinely apply in any laboratory, as opposed to the assessment of pronuclear morphology (Tesarik and Greco, 1999; Scott et al., 2000; Arroyo et al., 2007; Nicoli et al., 2013). Furthermore, with time-lapse methodologies, early cleavage is easier to assess (Rubio et al., 2012; Chamayou et al., 2013). Early cleavage has been related to embryo quality at day 2 (Racowsky et al., 2009), day 3 (Neuber et al., 2003) and embryo development up to the blastocyst stage (Rehman et al., 2007).

Early cleavage assessment has proved to have a predictive value in pregnancy rates after conventional IVF (Edwards et al., 1984; Shoukir et al., 1997) or intracytoplasmic sperm injection (ICSI) (Sakkas et al., 1998; Bos-Mikich et al., 2001; Lundin et al., 2001; Fenwick et al., 2002). Studies on single embryo transfer (SET) suggest that both early cleavage and the embryo quality score should be

taken into account in order to select an embryo with a high implantation potential (Salumets et al., 2003; Van Montfort et al., 2004; De las Heras et al., 2009). Giorgetti et al. (2007) proposed to use the predictive value of early cleavage to improve the selection of suboptimal quality embryos based on embryo morphology thus allowing SET cycles without reducing pregnancy rates. Moreover, with the introduction of time-lapse video cinematography in laboratory routines for embryo evaluation, morphokinetic parameters are becoming more relevant. The more relevant criteria described for the selection of good quality embryos are directly related to early cleavage corresponding to the time of first division and the duration of the different cell cycles (Meseguer et al., 2011).

The relationship between embryo quality and chromosomal abnormalities has been described at different stages of development. At day 1, different patterns of pronuclear scoring have been related to higher incidence of chromosomal abnormalities (Coskun et al., 2003; Arroyo et al., 2010). At day 2 and day 3 of embryo development, different morphological parameters and/or slow/arrested development present higher occurrence of abnormalities (Munné, 2006; Fragouli et al., 2013).

Data of this study are based on the static observation of early cleavage at 26–27 h post-insemination and FISH analysis of the embryos. The results presented will be useful for those laboratories that still do not have the time-lapse technology and comparative genomic hybridization arrays (aCGH) incorporated on their routine equipment. The purpose of this study is to analyze the possible association between the occurrence of early cleavage and chromosome constitution of embryos from a preimplantation genetic screening/Diagnosis (PGS/PGD) program, embryo quality at day 2 and implantation potential.

Material and methods

Patients

A total of 96 cycles corresponding to 86 couples undergoing an IVF cycle with PGS/PGD were included in this retrospective study. Indications for 83 PGS cases were male factor (31), repeated implantation failure (25), recurrent miscarriage (19) and X-linked genetic diseases (8); 13 PGD cycles were including balanced translocation, 12 of which were of male origin and 1 of female origin.

Ovarian stimulation (Barri et al., 2002) and laboratory procedures for gamete and embryo handling have been previously described (Arroyo et al., 2007). In all cases embryos were obtained by ICSI, cultured and biopsied according to our standard protocols.

Embryo scoring

Embryos were scored using an inverted microscope with Nomarski differential contrast optics at a magnification of 400 $\times$. Time of examination was minimized in order to preserve temperature and pH medium, which may affect subsequent embryo development, with timings adequate as to the routine of the laboratory.

Embryos were evaluated at 26–27 h (day 1) after ICSI and 3 groups were established: 2 pronuclei (2PN) still visible, absence of PN (No PN) or cleaved embryos (EC). In the EC group, symmetry, fragmentation and multinucleation of blastomeres at first division were assessed. Embryos no early-cleaved (NEC) group was comprised by the two categories No PN and 2PN. Embryo morphology was evaluated 40–44 h (day 2) after ICSI. Embryos at day 2 of development were scored according to morphological parameters such as cell number, symmetry, percentage of cytoplasmic fragmentation and blastomeres multinucleation. Two groups were considered: good morphology group (GM) which included embryos with at least 4 non-multinucleated blastomeres, symmetric or not, and $\leq 25\%$ fragmentation, and poor morphology group (PM) which comprised embryos with either < 4 symmetric or not blastomeres and/or at least one multinucleated blastomere and/or $\geq 30\%$ fragmentation (Arroyo et al., 2007).

Embryo biopsy and genetic analysis

Blastomere biopsy was performed on day 3 on embryos presenting at least 5 blastomeres and $\leq 35\%$ cytoplasmic fragmentation (Boada et al., 1998). Arrested embryos and those presenting $> 35\%$ cytoplasmic fragmentation were not biopsied. Embryo biopsy was performed as previously described (Boada et al., 1998). For the multicolor fluorescent in situ hybridization (FISH) analysis, specific DNA probes for chromosomes X, Y, 13, 15, 16, 18, 21 and 22 were used in a two-step protocol (Vidal et al., 1998). An embryo was considered to be aneuploid when the number of copies of any of these chromosomes was abnormal. An embryo was considered to be polyploid if it had more than two copies of all the chromosomes analyzed.

For PGD cycles involving translocation carriers, embryos were analyzed by FISH using specific probes for the

chromosomes involved in these chromosome reorganizations. Embryos from which no diagnosis was obtained were excluded from this study.

Biopsied embryos were kept in culture until day 5, when the embryos diagnosed as normal/balanced and presenting adequate development were transferred.

Clinical implantation and clinical pregnancy were defined by the presence of a gestational sac with heartbeat.

Independent Review Board approval was obtained.

Statistical analysis

Qualitative variables were compared using the 2-test or Fisher's exact test, with a significance level of $p \leq 0.05$.

Results

Cycles

The 96 cycles were distributed according to the fact whether early cleavage was observed in at least one embryo (ECpos) or in none (ECneg). Early cleavage assessment was performed at 26.9 ± 1.2 h after ICSI.

No significant differences with regard to patient's age, estradiol levels and number of follicles on the day of HCG administration were found among ECpos and ECneg groups. No statistically significant differences in the number of metaphase II oocytes were observed between these groups (Table 1).

The mean of the number of embryos transferred was 1.64 in the ECpos group, 2.04 in the ECneg and 1.57 in Mixed group (n.s.).

Overall implantation rate, pregnancy rate (Table 2) and live birth rate per cycle were 13.6%, 23.9% and 17.3%, respectively. When considering transferred embryos, three groups were established: a group in which all embryos presented early cleavage (ECpos) (32.8% cycles), another in which no early cleavage (ECneg) embryos were transferred (33.6% cycles) and a mixed group, in which some embryos were EC and some ECneg (Mixed) (33.6% cycles). ECneg group was divided in two categories depending on whether No PN was observed or they still presented 2PN. Statistically significant differences in the implantation rate among ECpos, ECneg and Mixed groups were observed (28.3%, 2.1% and 10.6%, respectively; ECpos vs. ECneg, $p < 0.001$; ECpos vs. Mixed $p < 0.05$; Table 2). When pregnancy rates were compared, statistically significant differences were observed only between EC and ECneg, the results being 35.7% vs. 6.7%; $p < 0.001$ (Table 2).

The overall incidence of EC in at least one embryo in all cycles was 83.3%. Among 83 cycles corresponding to a PGS indication, early cleavage was observed in 83.1% cycles while from 13 cycles with a PGD indication 84.6% cycles presented EC.

Embryos

Embryo development

Of 595 embryos studied, 37.2% exhibited EC. Embryos were analyzed separately according to PGS/PGD indication: 516 embryos corresponded to PGS and 79 were from PGD cycles.

Table 1 Patient characteristics according to early cleavage presentation (ECpos) or no early cleavage presentation (ECneg) of embryos.

	Patients	Age	E2 (pg/ml)	Follicles	MII
ECpos	79	37.5 ± 3.9	3000.8 ± 968.4	13.9 ± 4.4	14.7 ± 6.3
ECneg	17	40 ± 3.3	3161.7 ± 1079.3	14.2 ± 4.5	14.7 ± 6.9

PGS group. Of 516 analyzed embryos, 40.7% exhibited EC compared to 27.9% that no longer showed PN and 31.4% that still presented 2PN. Among the EC group, 66.2% embryos presented symmetric blastomeres (Table 3) and only 24.8% had fragmentation. The incidence of multinucleation at the time of early cleavage assessment was 3.3% but due to this low percentage no statistical analysis could be performed.

Regarding embryo quality on day 2, 41.9% of all embryos had good morphology. There were statistically significant differences between EC group and no early cleavage (NEC) group (71.4% vs. 47.7%; $p < 0.0001$). In the EC group, when the two blastomeres from the first cleavage division were symmetrical, significant differences were observed among good morphology embryos when compared to non-symmetrical cleavage (76.3% vs. 62%; $p < 0.05$). Differences were also observed in embryo quality on day 2 when EC embryos were compared, both with the No PN group (55.6%) and with 2 PN group (40.7%) ($p < 0.05$) (Table 3).

Overall blastocyst rate in PGS cycles was 33.7%, showing differences when comparing embryos presenting EC, with a higher blastocyst rate than that of NEC embryos (43.3% vs. 27.1%; $p < 0.001$).

No statistically significant differences were found in the blastocyst rate with regard to symmetry of division at the time of early cleavage assessment (Table 3).

PGD group. A total of 79 embryos were analyzed in this group. A total of 54.4% point four percent of the embryos exhibited EC, 26.6% did not have visible PN and 19% still showed 2PN. These differences were not statistically significant (Table 4).

Good morphology was found in 75.9% of the embryos. No statistically significant differences were observed concerning embryo quality according to early cleavage status or development until the blastocyst stage (Table 4).

When early cleavage characteristics were analyzed (symmetry of blastomeres or fragmentation) no differences were observed in embryo quality either (Table 4).

Chromosome constitution

PGS group. A total of 68.4% of the studied embryos were chromosomically abnormal. Early-cleaved embryos exhibited a lower rate of chromosome abnormalities than NEC embryos ($p = 0.0035$), and a lower rate when compared to the No PN and the 2PN group (61%, 69.4% and 77.2%, respectively; $p < 0.05$) (Table 5).

The most frequent chromosome abnormalities were aneuploidies, representing 92.9%. A total of 36.9% of aneuploid embryos presented EC, whereas the remaining 63.1% corresponded to NEC embryos ($p = 0.026$); among this group, 27.1% corresponded to the No PN group and 36% to 2PN group, these differences being statistically significant when compared to EC group ($p < 0.05$).

As for polyploid embryos, 23.1% were EC, 53.8% did not present PN and 23.1% presented 2PN. Although these differences were not statistically significant, the low number of cases in each group needs to be considered.

When the characteristics of early cleavage, such as symmetry of blastomeres or fragmentation in EC embryos were analyzed, no statistically significant differences were observed in chromosome constitution (Table 5).

PGD group. A total of 70.9% embryos from this group were chromosomally abnormal. Early-cleaved embryos exhibited the same rate of chromosome abnormalities as NEC embryos, whereas differences were observed when compared to the No PN and the 2PN groups (69.8%, 76.2% and 66.7% respectively; $p < 0.05$) (Table 5).

When the characteristics of early cleavage, such as symmetry of blastomeres or fragmentation in EC embryos, were

Table 2 Implantation and pregnancy rates by transferred embryos.

	Implanted embryos	Transferred embryos	Implantation rate	N° transfers	Pregnancies	Pregnancy rate/transfer
ECpos	13	46	28.3 ^{a,b}	28	10	35.7 ^{d,e}
ECneg	1	47	2.1 ^{a,c}	30	2	6.7 ^{d,f}
No PN	0	25	0	15	0	0
2PN	1	22	4.6	15	2	13.3
Mixed	5	47	10.6 ^{b,c}	23	6	26.1 ^{e,f}
Total	19	140	13.6	71	17	23.9

^a Implantation rate (IR): ECpos vs. ECneg $p < 0.001$.

^b IR: ECpos vs. Mixed $p < 0.05$.

^c IR: ECneg vs. Mixed n.s.

^d Pregnancy Rate/Transfer (PR/T): ECpos vs. ECneg $p < 0.001$

^e PR/T: ECpos vs. Mixed, n.s.

^f PR/T: ECpos vs. Mixed, n.s.

Table 3 Characteristics of PGS embryos.

EC	<i>n</i>	%	Good morphology	%	Blastocyst	%
EC	210	40.7	150	71.4 ^{a,b}	91	43.3 ^{d,e,f,g}
EC symmetry	139	66.2	106	76.3 ^c	62	44 ⁱ
No-EC symmetry	71	33.8	44	62 ^c	29	40.8 ⁱ
NEC	306	59.3	146	47.7 ^a	83	27.1 ^d
No PN	144	27.9	80	55.6 ^b	42	29.2 ^{e,f,h}
2 PN	162	31.4	66	40.7 ^b	41	25.3 ^{e,g,h}
Total	516	100	296	57.4	174	33.7

^a Good morphology embryos (GM) on Day 2: EC vs. NEC $p < 0.0001$.

^b GM embryos: EC vs. No PN and 2 PN $p < 0.05$.

^c GM embryos: Symmetry on EC vs. No Symmetry $p = 0.0448$.

^d Blastocyst rate: EC vs. NEC $p = 0.0002$.

^e Blastocyst rate: EC vs. No PN and 2 PN $p < 0.05$.

^f Blastocyst rate: EC vs. No PN $p < 0.05$.

^g Blastocyst rate: EC vs. 2 PN $p < 0.05$.

^h Blastocyst rate: No PN vs. 2 PN n.s.

ⁱ Blastocyst rate: Symmetry on EC vs. No Symmetry n.s.

analyzed, no differences were observed in chromosome constitution either (Table 5).

Discussion

Assessment of early cleavage has been a useful tool to determine embryo quality allowing a reduction on the number of embryos transferred in order to reduce multiple pregnancy rates (Emiliani et al., 2006). The first studies on early cleavage were rather confusing because they reported mixed transfers of EC and NEC embryos (Shoukir et al., 1997; Sakkas et al., 1998). However, homogeneous transfers of EC embryos were soon published reporting better implantation and pregnancy rates (Lundin et al., 2001; Salumets et al., 2003; Ciray et al., 2004; Van Montfoort et al., 2004; Emiliani et al., 2006; Giorgetti et al., 2006; Dumont, 2008).

Inclusion of the early cleavage criterion on graduated embryo scores has also played an important role (Fisch et al., 2001; Neuber et al., 2003; Brezinova et al., 2009; Racowsky

et al., 2009). In this sense, using a time-lapse photography system, Lemmen et al. (2008) found better implantation rates in embryos with an early-PN disappearance, early cleavage and good morphology on day 2.

Evaluation of embryo morphology at first cleavage stage in a PGS program may seem to be a tool of little relevance given the additional information we obtain from the genetic analysis, but it may provide an additional argument when deciding the convenience of performing the PGS.

The assessment of early cleavage in IVF lab routines depends on the features of every program. It is critical to determine the appropriate moment for checking the first cell division, since this can take place as early as 22 h after insemination (Sakkas et al., 2001; Lemmen et al., 2008). If the observation is performed too soon, the number of zygotes that have already divided could still be too low, but if it is performed too late, all the embryos will have divided and it will be impossible to distinguish those that divided first, which therefore have a better prognosis (Na et al., 2008). The frequency of early cleavage varies from 10% to

Table 4 Characteristics of PGD embryos.

EC	N	%	Good morphology	%	Blastocyst	%
EC	43	54.4	36	83.7 ^{a,b}	15	34.9 ^{d,e}
EC symmetry	34	79.1	29	85.3 ^c	14	41.2 ^f
No EC symmetry	9	20.9	7	77.8 ^c	14	31.1 ^f
NEC	36	45.6	24	66.7 ^a	13	36.1 ^d
No PN	21	26.6	15	71.4 ^b	7	33.3 ^e
2 PN	15	19	9	60 ^b	6	40 ^e
Total	79	100	60	75.9	28	35.4

^a Good morphology embryos (GM) on Day 2: EC vs. NEC n.s.

^b GM embryos: EC vs. No PN and 2 PN n.s.

^c GM embryos: Symmetry on EC vs. No Symmetry, n.s.

^d Blastocyst rate: EC vs. NEC n.s.

^e Blastocyst rate: EC vs. No PN and 2 PN n.s.

^f Blastocyst rate: Symmetry on EC vs. No Symmetry n.s.

Table 5 Chromosome constitution of PGS and PGD embryos.

EC	PGS			PGD			Total		
	N	Chromosomically abnormal (%)		N	Chromosomically abnormal (%)		N	Chromosomically abnormal (%)	
EC	210	128	61 ^{a,b,c,d}	43	30	69.8 ^{g,h}	253	158	62.5 ^{i,j,k,l}
EC symmetry	139	81	58.3 ^f	34	23	67.6	173	104	60.1 ^m
No EC symmetry	71	47	66.2 ^f	9	7	77.8	80	54	67.5 ^m
NEC	306	225	73.5 ^a	36	26	72.2 ^g	342	251	73.4 ⁱ
No PN	144	100	69.4 ^{b,c,e}	21	16	76.2 ^h	165	116	70.3 ^{j,k,n}
2 PN	162	125	77.2 ^{b,d,e}	15	10	66.7 ^h	177	135	76.3 ^{j,l,n}
Total	516	353	68.4	79	56	70.9	595	409	68.7

^a PGS: Chromosomically abnormal EC vs. NEC $p=0.0035$.

^b PGS: Chromosomically abnormal EC vs. No PN and 2PN $p<0.05$.

^c PGS: Chromosomically abnormal EC vs. No PN n.s.

^d PGS: Chromosomically abnormal EC vs. 2PN $p<0.001$

^e PGS: Chromosomically abnormal No PN vs. 2PN n.s.

^f PGS: Symmetry on EC vs. No EC Symmetry, n.s.

^g Translocations: Chromosomically abnormal EC vs. NEC n.s.

^h Translocations: Chromosomically abnormal EC vs. No PN and 2PN $p<0.05$.

ⁱ Total: Chromosomically abnormal EC vs. NEC $p=0.0058$.

^j Total: Chromosomically abnormal EC vs. No PN and 2PN $p=0.0086$.

^k Total: Chromosomically abnormal EC vs. No PN n.s.

^l Total: Chromosomically abnormal EC vs. 2PN $p=0.0035$.

^m Total: Symmetry on EC vs. No EC Symmetry, n.s.

ⁿ Total: Chromosomically abnormal No PN vs. 2PN n.s.

60% of embryos, depending greatly on the time-points of observation (Shoukir et al., 1997). Bos-Mikich et al., 2001, observed embryos at 25, 27 and 29 h and define the best time-point for early cleavage determination between 25 and 27 h. The latest publications using time-lapse methodology reported that the good prognosis time-point for the first division begins about 24 h post-insemination, lasting until 28 h (Meseguer et al., 2011). This variation in the time of the first cell division is believed to depend on culture conditions as well as on intrinsic oocyte and sperm factors (Lundin et al., 2001).

In our results we found a relatively high frequency of early cleavage (40.7% in PGS and 54.4% in PGD) compared to the frequencies described in the literature (Lemmen et al., 2008; Racowsky et al., 2009). An explanation would be the fact that it is a biased population of good-quality embryos suitable for biopsy in which a high percentage of early cleavage could be thus expected. This explanation is supported by the fact that the general data of our IVF program show a lower overall early cleavage rate (35.8%; De las Heras et al., 2009). Moreover, the time of observation in this study (26.9 h) as well as in the routine of our IVF program (26.7 h) (De las Heras et al., 2009) is within the upper range of the time frame normally used.

In this study, no statistically significant differences in patients' characteristics were found between groups according to the presence of EC embryos (ECpos) or not (ECneg), demonstrating the comparability of these groups. Patients from both groups were considered at advanced maternal age (37.5 and 40 years old, respectively), and both displayed good stimulation response, which yielded a sufficient number of embryos for a PGS/PGD cycle. Given this

similarity, we can assume that the differences observed in the implantation rate between ECpos and ECneg cycles were not attributable to the patients' response to the ovarian stimulation.

An association between early cleavage and embryo quality has been found in the PGS group. This result agrees with other reports in conventional IVF programs, in which differences between EC and NEC embryos have been found, specifically in terms of the presence of PN and embryo quality at day 2 (Neuber et al., 2003) and day 3 (Chen et al., 2003; Lee et al., 2012). Additionally, our results confirm that assessing the early disappearance of PN before the first division is also a good prognosis factor, as has been previously described in IVF patients (Fancsoviets et al., 2005).

Among the group of EC embryos in PGS, we found a connection between the symmetry of blastomeres at first cleavage and embryo quality at day 2 as previously described in IVF (Ciray et al., 2006). Moreover, studies on time-lapse videocinematography have demonstrated that direct cleavage from 1 to 3 cells on the first cell cycle is associated with a lower implantation potential (Rubio et al., 2012). With respect to fragmentation in early cleavage and embryo quality, no connection was observed possibly because the mean percentage of fragmentation in the group of embryos studied is about 12%, lower than the 20% described in publications analyzing IVF cycles where this correlation was observed (Fisch et al., 2001). As for embryo developmental capacity, we found a higher blastocyst rate among EC embryos in the PGS group only. The results are similar to those described in earlier publications in which no biopsy is performed (Fenwick et al., 2002; Guerif et al., 2006), indicating that this procedure does not significantly affect

embryo developmental capacity. Moreover, the fact that EC embryos presented better blastocyst rate to NEC embryos supported the idea that EC is a good prognosis parameter of embryo development. Morphokinetic parameters analyzed using time-lapse technology demonstrated that early cleavage is related to development to the blastocyst stage that also depends on media and culture conditions (Cruz et al., 2012; Kirkegaard et al., 2013).

Regarding the association between embryo morphology and chromosome constitution, it is well known that they do not always match (Sandalinas et al., 2001). Our results in terms of aneuploidy and polyploidy percentage of the embryos analyzed are comparable to those described in earlier studies (Magli et al., 2007) referring to those embryos that reached 6–8 cell stage. Although these studies showed higher aneuploidy and polyploidy rates among embryos arrested on day 2, it should be noted that all embryos included in our study were biopsied; therefore they all overcame the day-2 blockage.

As for early cleavage assessment (i.e. first division, absence or presence of 2PN), we did not find any previous study analyzing its relationship with the chromosome constitution of the embryo. Our results showed a direct association between embryo stage at 25–27 h post ICSI and chromosome abnormalities on day 3. A decreasing proportion of chromosome abnormalities were observed among 2PN, No PN and EC embryos in PGS. This connection should be compared to an extrapolation derived from the results described in earlier publications between embryo quality and chromosome constitution (Munné, 2006). A relationship between dominance of a single blastomere and polyploidy has been described (Munné et al., 1994), as well as between aneuploidy and dysmorphisms on day 3, such as fragmentation (Magli et al., 2001) and multinucleation (Kligman et al., 1996). Moreover, embryos presenting blastomere asymmetry as the single dysmorphism have more post-meiotic abnormalities, such as mosaicism, polyploidy and haploidy than symmetric ones, although their aneuploidy rate is similar (Hardarson et al., 2001). Recent studies on time-lapse videocinematography and aCGH screening show a relationship between the time of the first or second division and the origin of the chromosome abnormality (Chavez et al., 2012). In this sense, there is a delay on the time of the first division in embryos with meiotic errors, whereas the delay occurs in the second division in embryos with mitotic errors. Moreover, an asynchrony between blastomeres' division was observed that was related to chromosome abnormalities. Due to the fact that the presence of triploidies is undetectable with CGH, these authors confirm their results with FISH analysis. In the group of embryos with meiotic chromosomal errors a higher incidence of cytoplasmic fragmentation at first division was observed.

As for translocation carriers of PGD group, our results showed an association between the moment of first cleavage and chromosome constitution. Fiorentino et al. (2011) have described normal/balanced embryos for the translocation with higher incidences of aneuploidies for other chromosomes, probably due to the interchromosomal effect. In our study, translocations were mainly of male origin with patients presenting altered semen parameters, which suggested us a possible interchromosomal effect (Anton et al., 2011). However, certain unbalanced products of the

translocation may have such a low degree of partial aneuploidy that they do not affect cell division. This agrees with data where no relationship between embryo quality in early stages of development and chromosomal abnormalities were found in embryos from translocation carriers (Ménézo et al., 2001).

It should be noted that the genetic analysis of the embryos included in our study was performed using the FISH technique, and only the chromosomes involved in the reorganization were analyzed. This situation could change the relationship observed between chromosome constitution and early cleavage. Moreover, other preimplantation genetic diagnosis strategies allowing simultaneous analysis of all chromosomes (m-CGH, a-CGH or aSNP) revealing more abnormalities could emphasize these results.

Finally, indications for a couple to undergo a PGS are still controversial (Mastenbroek et al., 2011) and in many cases the decision depends on the number and status of the embryos available for biopsy on day 3. Early cleavage assessment could help in this decision. According to our results, although EC embryos present a lower chromosome abnormality rate, the percentage of abnormal embryos among them is still too high (61%). Therefore, using early cleavage assessment as a determinant criterion for embryo selection seems to be insufficient and we definitely do not believe that it could replace preimplantation genetic diagnosis. Data of this study are based on the observation of early cleavage at 26 h post-insemination and FISH analysis of the embryos. The results presented will be useful for those laboratories that still do not have access to tools like time-lapse technology and aCGH.

Early cleavage has proved to associate with embryo quality, with the capacity to develop up to blastocyst stage as well as with euploid chromosome constitution. The new embryo morphokinetic and preimplantation genetic diagnosis technologies will certainly broaden the information available, but for the moment a simple procedure such as early cleavage assessment can offer valuable information that could contribute to the difficult process for the selection of embryos for transfer and/or cryopreservation.

Conflict of interest

The authors declare no conflict of interest.

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