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The effect of pronuclear morphology on early development and chromosomal abnormalities in cleavage-stage embryos

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BACKGROUND: Pronuclear (PN) zygote morphology has been proposed as a useful tool for selecting the best embryos for transfer. METHODS: PN morphology was recorded in 888 zygotes and classified according to similar/different PN size [groups A (n = 816) and B (n = 72)] and to the number, distribution and synchrony of nucleolar precursor bodies (NPB): subgroup I, pronuclei with 3–4 polarized NPB; subgroup II, 5–7 synchronic polarized NPB or 7–10 NPB distributed randomly; and subgroup III, morphologies other than those of groups I or II. Embryo development and chromosomal abnormalities were evaluated for each PN pattern. RESULTS: In patients aged ⩽37 years, the number of zygotes reaching morula and blastocyst stage was significantly (P = 0.0003) higher in group A than in group B. In group A, the incidence of chromosomal abnormalities was significantly (P = 0.0247) lower than in group B, and significant differences were observed when pattern AI was compared with pattern AII (P = 0.0280), AIII (P = 0.0024), BIII (P = 0.0077) and total B (P = 0.0247). In patients aged >37 years, statistical differences among groups were not observed. CONCLUSIONS: In patients aged ⩽37 years, zygotes with similar PN size and with polarized NPB present the best prognosis based on embryo development and the incidence of chromosomal abnormalities, whereas in patients aged >37 years, this correlation does not exist.

Key words: age/chromosomal abnormalities/ pronuclear morphology/nucleolar precursor bodies

Introduction

Approximately 30% of human embryos generated by IVF treatments present an abnormal chromosome constitution (Márquez *et al*., 2000; Rubio *et al*., 2003). This percentage may reach 60% in embryos originating from poor-prognosis IVF patients such as low responders, women of advanced age and patients with a history of IVF failure (Magli *et al*., 1998; Gianaroli *et al*., 1999) or recurrent miscarriage (Pellicer *et al*., 1999; Rubio *et al*., 2003).

The morphology of human embryos is an important parameter that seems to be related to chromosome anomalies. It has been shown that dysmorphic and slow-developing or arrested embryos exhibit significantly more polyploidy and mosaicism than normally developing human embryos (Munné *et al*., 1995; Márquez *et al*., 2000). Increased incidences of aneuploidy and multinucleation have also been observed in embryos with a high degree of fragmentation and in those with irregular or unevenly sized blastomeres (Hardarson *et al*., 2001).

With regard to human embryo development, only ~25% of aneuploid embryos reach blastocyst stage compared with 62% of euploid embryos (Rubio *et al*., 2003). Interestingly, a low percentage of monosomies are found at blastocyst stage, and an important percentage of trisomic embryos achieve blastocyst stage, which confirms observations in spontaneous abortions (Sandalinas *et al*., 2001; Rubio *et al*., 2003). Some aneuploid human embryos do reach blastocyst stage, and the transfer of such abnormal embryos to the uterus may be in part responsible for the lack of pregnancy and implantation in some IVF patients. Efforts have been made to find an adequate criterion for selecting the best embryo for transfer, thereby reducing the number of embryos that are transferred without affecting success rates (Alikani *et al*., 2000). Thus, a recently introduced parameter in embryo selection is the pronuclear score. This is the assessment of pronuclear zygote morphology and the evaluation of the number and distribution of nucleolar precursor bodies (NPB). Embryo quality has also been correlated with the presence of a clear cortical zone (Payne *et al*., 1997). In this respect, one group (Scott and Smith, 1998) performed pronuclear embryo transfers and found that zygotes with perinuclear condensation were associated with higher implantation rates. In a recent report, these authors found that

cytoplasmic halo-negative zygotes displayed slow embryo development, poor morphology and lower blastocyst formation (Scott, 2003).

Embryos with a good pronuclear score (halo positive + PNs aligned + similar number and polarized NPB) have shown better implantation potential than embryos without this pronuclear pattern, both in retrospective (Payne *et al*., 1997; Scott and Smith, 1998) and prospective (Scott and Smith, 1998; Tesarik and Greco, 1999; Ludwig *et al*., 2000; Scott *et al*., 2000; Wittemer *et al*., 2000) studies. Furthermore, abnormal patterns of pronuclear morphology are correlated to a higher rate of cleavage arrest, as well as lower blastocyst development (Scott and Smith, 1998; Tesarik and Greco, 1999; Scott *et al*., 2000; Wittemer *et al*., 2000; Balaban *et al*., 2001; Fisch *et al*., 2001; De Placido *et al*., 2002; Rienzi *et al*., 2002; Zollner *et al*., 2002). Current studies have found that zygotes with different pronuclear sizes present a significantly higher incidence of both embryo cleavage arrest and mosaicism in day 3 embryos than do normal zygotes (Munné and Cohen, 1998; Sadowy *et al*., 1998) and lower developmental potential (Scott, 2003).

Since both the chromosomal status of the embryo (Sandalinas *et al*., 2001; Rubio *et al*., 2003) and pronuclear patterns seem to be related to embryo cleavage ability, a link may exist between the pronuclear score and chromosome constitution of human embryos. Therefore, in the present study the aim was to investigate whether a correlation existed between pronuclear morphology during the zygote stage and chromosome abnormalities in day 3 human embryos. Embryo development up to blastocyst stage was also studied with respect to pronuclear morphology patterns.

Materials and methods

Patients and protocols

Pronuclear morphology and embryo development were analysed in 888 zygotes from 69 patients with normal karyotypes undergoing 81 preimplantation genetic diagnosis (PGD) cycles between November 2000 and December 2001. Additionally, chromosomal assessment was performed on day 3 in 569 developing embryos derived from these zygotes. To eliminate age as a factor in the analysis, as it is known that aneuploidy increases with age, data were broken down into two groups according to maternal age ⩽37 years (53 cycles) and >37 years (28 cycles).

Indications for PGD were as follows: 31 couples with recurrent miscarriage (RM) of unknown aetiology, 32 couples with repetitive implantation failure (IF) following IVF or ICSI, 16 couples with aneuploidy screening (AS) due to advanced maternal age or mixed causes, and two couples with a high incidence of chromosomal abnormalities after fluorescence in-situ hybridization (FISH) analysis in the spermatozoa. Stimulation, oocyte retrieval and ICSI procedures were performed as described previously (Pellicer *et al*., 1999).

Assessment of zygote and embryo morphology

Pronuclear zygote morphology was assessed at 16–18 h post-ICSI at ×40 magnification under an inverted microscope. Two groups were formed based on pronuclei size: those of equal or very similar size (group A); and those of different sizes (group B). In each group, zygotes were subdivided into three categories according to the number, distribution and synchrony of NPB (Figure 1): subgroup I, pronuclei with 3–4 polarized NPB; subgroup II, 5–7 synchronic polarized NPB or 7–10 NPB distributed randomly throughout the pronucleus; and subgroup III, morphologies other than those of groups I or II (asynchronic NPB polarization, alignment of more than 7 NPB at the point of contact of the two pronuclei, a difference in more than three NPB between pronuclei, random distribution of ⩽4 NPB in both pronuclei). The presence of a cytoplasmic halo was also evaluated in 537 zygotes, classifying zygotes as halo-positive when they showed a perinuclear condensation of the cytoplasm, and halo-negative when this polarization of the cytoplasm did not exist.

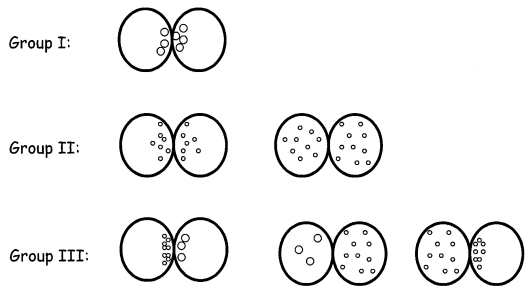

Figure 1. Pronuclear patterns according to nucleolar precursor bodies (NPB) number and distribution. Refer to text for group definitions.

Embryo morphology was evaluated on days 2, 3 and 4, taking into account the number, symmetry and granularity of blastomeres, type and percentage of fragmentation, presence of multinucleated blastomeres and degree of compaction (previously described). Day 5 human embryos were scored according to the expansion of the blastocele cavity and the number and integrity of both the inner cell mass and trophoectoderm cells, as described previously (Alikani *et al*., 2000).

Embryo coculture

After removal of the cumulus, oocytes, zygotes and embryos were cultured in 50 µl microdroplets of IVF medium (Scandinavian IVF; Gothenburg, Sweden) until day 2. From day 2 to day 5, embryos were kept individually in coculture with epithelial endometrial cells (EEC) as follows: on day 2, when at the 2- to 4-cell stage, embryos were placed in coculture in the presence of EEC with 1 ml IVF:CCM (1:1) (Scandinavian IVF); on days 3, 4 and 5, embryos were cocultured with 1 ml CCM. Ultrasound-guided embryo transfers were performed on day 5 using either Wallace (SIMS Portex Limited, Kent, UK) or Gynétics (Gynétics Medical Products, Hamont-Achel, Belgium) catheters.

Embryo biopsy and FISH protocol

Embryo biopsy was performed on day 3 developing embryos with ⩾5 nucleated blastomeres and a ⩽25% degree of fragmentation. In embryos with ⩾7 cells, two blastomeres were biopsied and in embryos with <7 cells only one blastomere was retrieved. Two cells were analysed in 318 embryos, and in the remaining 251 embryos one cell was analysed. Mosaic embryos were classified as those having different FISH results when two cells were analysed (318 embryos). The risk of FISH errors was assumed, but the risk was considered to be homogeneously distributed in all groups and did not interfere in the statistical analysis of the results.

For the biopsy, embryos were placed on a droplet containing Ca^{2+}- and Mg^{2+}-free medium (EB-10; Scandinavian IVF). Tyrode's solution (ZD-10; Scandinavian IVF) was used to perforate the zona pellucida. One or two blastomeres were withdrawn with a bevelled aspiration pipette and individually fixed under an inverted microscope with methanol:acetic acid (3:1), using a slightly modified Tarkowski's

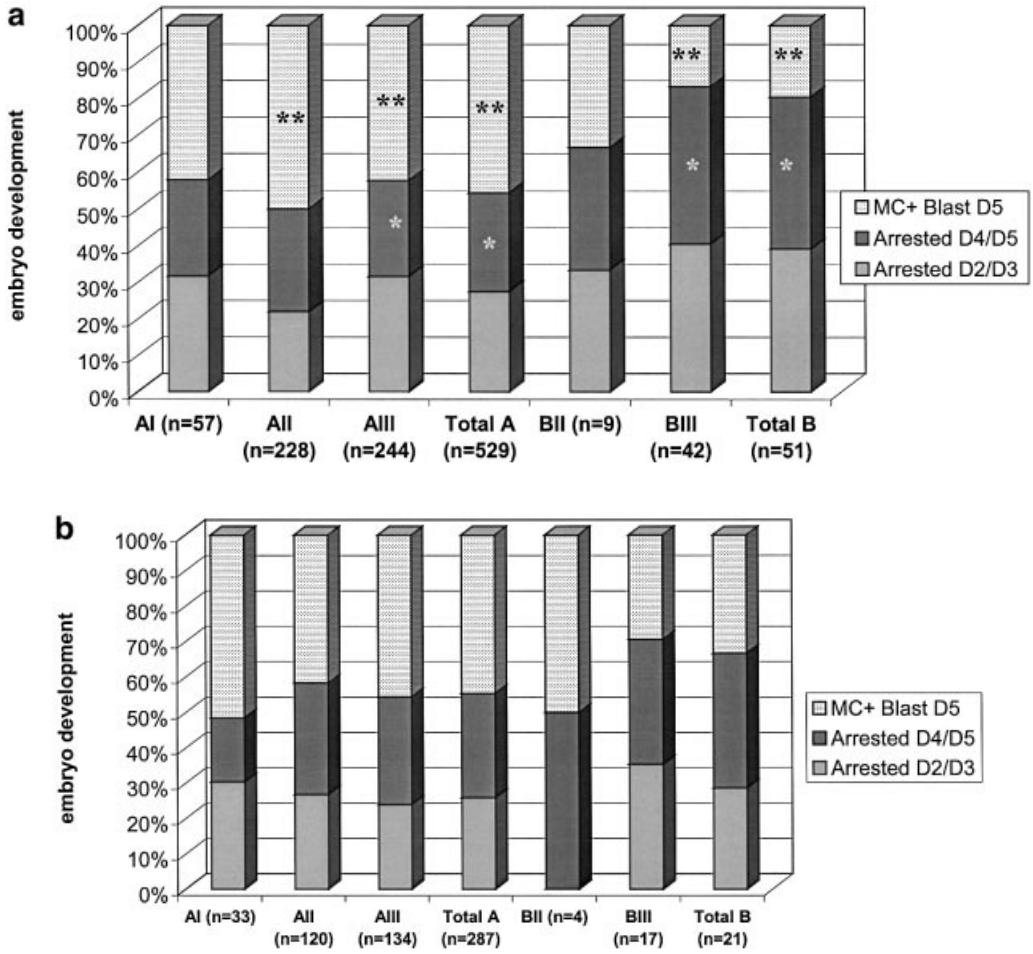

Figure 2. Embryo development according to PN patterns in patients aged ⩽37 years (a) and aged >37 years (b). *Embryos arrested on days 4/5: total A versus total B, $P = 0.0493$; AIII versus BIII, $P = 0.0408$. **Compacted morula/blastocyst on day 5: total A versus total B, $P = 0.0003$; AI versus BIII, $P = 0.0085$; AII versus BIII, $P < 0.0001$; AIII versus BIII, $P = 0.0017$.

protocol without hypotonic pretreatment (Tarkowski, 1966; Rubio *et al*., 2003).

In all blastomeres from the 569 day 3 embryos, seven chromosomes were analysed using FISH in three steps. A first hybridization round was performed using locus-specific probes for chromosomes 13 and 21. In a second round, after signal elimination, blastomeres were hybridized with a centromeric probe for chromosome 16 and a locus-specific probe for chromosome 22. Finally, in a third round, a triple FISH was performed with centromeric probes for chromosomes X, Y and 18 (all probes commercially available from Vysis Inc., Downers Grove, IL, USA).

The percentage of abnormal embryos in each group was estimated as the number of affected embryos divided by the number of embryos analysed, regardless of the probe employed. Embryos were classified as normal, abnormal, mosaic (two blastomeres from the same embryo displaying different results) and multinucleated (two or more nuclei/blastomere). To analyse embryo development, the following groups were established: embryos arrested at days 2 or 3; embryos arrested at days 4 or 5 without compaction or cavitation; and embryos at morula or blastocyst stage on day 5. Abnormal embryos were classified as carriers of autosomal monosomies, carriers of monosomy X, carriers of autosomal trisomies and trisomies for sex chromosomes, those with combined monosomy and trisomy and haploid, triploid, tetraploid and mosaic embryos.

Statistical analysis

For statistical comparison between groups, chi-square analysis and Fisher's exact test were used to compare the percentages of chromosomally abnormal embryos and arrested embryos at different stages. A P-value <0.05 was considered statistically significant. The statistical analysis was carried out using the Graphpad Instat v. 2.05a package (Graphpad Software, San Diego CA, USA).

Results

A total of 888 zygotes was analysed, these being distributed in five categories: AI, AII, AIII, BII and BIII. None corresponded with group BI (different PN size and 3–4 polarized NPB). A total of 816 zygotes was included in group A (91.9%) and 72 in group B (8.1%).

The relationship between zygote patterns and early embryonic development until day 5 is detailed in Figure 2a and b. In patients aged ⩽37 years (Figure 2a), the percentage of zygotes arrested on days 2 and 3 was not statistically different in the five PN patterns. Blastocyst/morula rates showed statistical differences between groups AI and BIII (42.1 versus 16.7; $P = 0.0085$), AII and BIII (50.0 versus 16.7; $P < 0.0001$), and AIII and BIII (42.2 versus 16.7; $P = 0.0017$). The number of zygotes

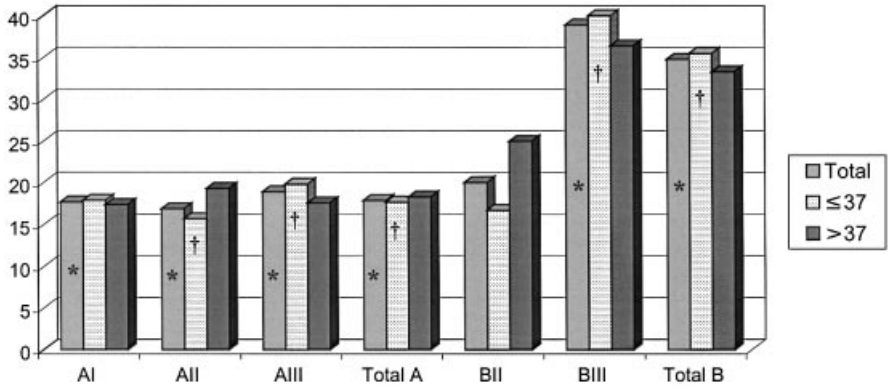

Figure 3. Embryos with slow embryo development according to PN patterns. *Total number of zygotes: total A versus total B, $P = 0.01$; AI versus BIII, $P = 0.03$; AII versus BIII, $P = 0.0035$; AIII versus BIII, $P = 0.0370$. †Zygotes from patients ⩽37 years: total A versus total B, $P = 0.0288$; AII versus BIII, $P = 0.0106$; AIII versus BIII, $P = 0.0370$.

Table I. Incidence of chromosomal abnormalities identified in embryos from women aged ⩽37 years

Embryo stage	Group/subgroup						
	AI	AII	AIII	Total A	BII	BIII	Total B
No. of 2PN	57	228	244	529	9	42	51
No. of embryos analysed	35	154	156	345	5	18	23
Abnormal embryos (%)	18 (51.4)[a,b,c,d]	110 (71.5)[a]	111 (72.7)[b]	376 (69.3)	3 (60.0)	16 (88.9)[c]	19 (73.1)[d]

[a]AI versus AII, $P = 0.0280$; [b]AI versus AIII, $P = 0.0024$; [c]AI versus BIII, $P = 0.0077$; [d]AI versus total B, $P = 0.0247$.

Table II. Incidence of chromosomal abnormalities identified in embryos from women >37 years of age

Embryo stage	Group/subgroup						
	AI	AII	AIII	Total A	BII	BIII	Total B
No. of 2PN	33	120	134	287	4	17	23
No. of embryos analysed	21	81	89	191	4	6	10
Abnormal embryos (%)	15 (71.4)	55 (67.9)	67 (75.3)	137 (71.7)	3 (75.0)	4 (66.7)	7 (70.0)

There were no statistically significant differences between groups.

reaching morula and blastocyst stage was greater in group A (45.6%) than in group B (19.6%) ($P = 0.0003$). The percentages of embryos arrested on days 4 and 5 varied significantly ($P = 0.0493$) between zygotes with similar pronuclear size (group A) and zygotes with unequal pronuclear size (group B) (27.4 versus 39.2). Statistical differences among groups were not observed in zygotes from couples aged >37 years (Figure 2b).

The incidence of slow-developing day 3 embryos is represented in Figure 3, with a similar distribution in the two age groups. Including the total number of zygotes analysed, statistical differences were observed between groups A and B ($P = 0.01$), AI versus BIII ($P = 0.03$), AII versus BIII ($P = 0.0035$) and AIII versus BIII ($P = 0.0093$). In the maternal group aged ⩽37 years, the percentage of embryos with delayed development was significantly different between groups: AII versus BIII ($P = 0.0106$) and AIII versus BIII ($P = 0.0370$). Statistical differences were not observed between AI versus BIII due to the lower number of embryos analysed in these two groups. Nevertheless, total group A (17.7) showed significant ($P = 0.0288$) differences compared with total group B (35.5). Statistical differences were not observed when the maternal age was >37 years (18.3 versus 33.3), although there was a trend towards an increased incidence of slow embryos in group B.

The correlation between PN patterns and multinucleated embryos on day 2 and day 3 was also evaluated. On day 2, similar percentages of multinucleated embryos were observed in each PN subgroup, with a total of 15.4% in group A and 18.5% in group B. However, on day 3, the percentage of multinucleated embryos from zygotes with different PN size (group B) was twice that of zygotes with similar PN size (group A) (10.9 versus 5.4%). These differences can be attributed mostly to the group of patients aged ⩽37 years (12.9 versus 4.9%), although these values did not reach statistical significance.

The incidence of chromosomally abnormal embryos is evaluated for each PN pattern, according to the maternal age

Table III. Description of chromosomal abnormalities found in day 3 embryos, according to the pronuclear pattern

Chromosomal abnormality	Group/subgroup						
	AI	AII	AIII	Total A	BII	BIII	Total B
Autosomal monosomy	11	59	58	128	0	8	8
Monosomy X	1	4	4	9	1	1	2
Autosomal monosomy/monosomy X	0	2	2	4	0	0	0
Autosomal trisomy	6	38	42	86	2	4	6
Trisomy for sex chrom.	0	2	2	4	0	0	0
Monosomy/trisomy	2	15	13	30	1	1	2
Haploid	3	8	7	18	0	2	2
Triploid	1	3	4	8	0	1	1
Tetraploid	1	4	4	9	0	0	0
Mosaic[a]	8/30	30/139	42/136	80/305	2/5	3/8	5/13

[a]Number of mosaic embryos/number of embryos with two cells analysed in each group.

Table IV. Embryo development and chromosomal abnormalities in cytoplasmic halo-positive and halo-negative zygotes

Embryo development/chromosomal abnormality	Age ⩽37 years		Age >37 years	
	Halo-positive	Halo-negative	Halo-positive	Halo-negative
No. of pattern A	245	56	147	36
Blastocyst day 5 (%)	84 (34.3)[a]	8 (14.3)[a]	52 (35.4)	8 (22.2)
Abnormal/analysed (%)	95/156 (60.9)	27/36 (75)	78/113 (69)	14/21 (66.7)
No. of pattern B	23	6	7	2
Blastocyst day 5 (%)	2 (8.7)	1 (16.7)	2 (28.6)	1 (50.0)
Abnormal/analysed (%)	5/6 (83.3)	4/5 (80)	3/3 (100)	1/1 (100)
Total	268	62	154	38
Blastocyst day 5 (%)	86 (32.1)[b]	9 (14.5)[b]	54 (35.1)	9 (23.7)
Abnormal/analysed (%)	100/162 (61.7)	31/41 (75.6)	81/116 (69.8)	15/22 (62.8)

[a]$P = 0.0035$. [b]$P = 0.0051$.

groups, in Tables I and II. A stronger correlation of PN patterns with chromosomal abnormalities was observed in patients aged ⩽37 years (Table I). Statistical differences were observed when pattern AI (51.4) was compared with pattern AII (71.5%, $P = 0.0280$), pattern AIII (72.7%, $P = 0.0024$), pattern BIII (88.9%, $P = 0.0077$) and total B (73.1%, $P = 0.0247$). When the maternal age was >37 years, the incidence of chromosomal abnormalities was independent of the pronuclear pattern (Table II). A detailed description of the chromosomal abnormalities found in each subgroup is shown in Table III.

The effect of a cytoplasmic halo was analysed as an independent parameter, with the identification of 422 halo-positive and 100 halo-negative zygotes (Table IV). In patients aged ⩽37 years, blastocyst rates in halo-positive zygotes were significantly ($P = 0.0051$) higher than those in halo-negative zygotes (32.1 versus 14.5%), while no differences were noted in patients aged >37 years. Insofar as the incidence of chromosomal abnormalities in day 3 embryos from halo-positive and halo-negative zygotes, statistical differences were not observed, though there was a slight difference in the percentage of chromosomally abnormal embryos in patients aged <37 years (61.7 and 75.6% respectively).

The significance of sperm quality in PN patterns was also evaluated. In patients aged ⩽37 years, the percentage of zygotes derived from normozoospermic sperm samples was significantly ($P = 0.0440$) higher in group A than in group B (27.4 versus 13.7%). Statistical differences based on NPB distribution were not observed, although the percentage of normozoospermic samples displaying AI pattern was twice as high as that displaying BIII pattern (31.6 versus 13.7%). No differences were observed between group A and group B in patients aged >37 years.

Discussion

The results of the present study, which was conducted in poor-prognosis patients, showed that zygotes with similar pronuclear size and with 3–4 polarized NPB present the best prognosis, based on embryo development and the low incidence of chromosomal abnormalities in day 3 embryos. The size of the pronuclei proved to be the factor which most affected embryo development from day 3 onwards. When both pronuclei were of a similar size, a higher percentage of compacted or cavitated embryos was found on day 5, and a lower percentage of multinucleation in slow-developing embryos on day 3. Interestingly, this observation only applied to patients aged ⩽37 years, and showed that the PN score could be considered a useful tool for selecting the best zygotes/embryos for transfer in this age group.

In patients aged >37 years, oocyte aging—with all that this implies—seems responsible for poor reproductive outcome. It is known that implantation rates decrease after the age of 37 years (Van Kooij *et al*., 1996), and age has been highlighted as the most influential factor in reproductive outcome affecting pregnancy and miscarriage rates (Ron-El *et al*., 2000; Spandorfer *et al*., 2000). The success of oocyte donation programmes in older patients has given credence to the idea that the endometrium retains normal receptivity (Remohí *et al*., 1997). On the other hand, a high incidence of chromosomal abnormalities has been observed in preimplantation embryos of patients aged over 37 years (Munné *et al*., 1995; Gianaroli *et al*., 1999; Pellicer *et al*., 1999). The incidence of non-disjunction of bivalent chromosomes during oogenesis has been shown to increase with age (Hassold and Sherman, 2000). The majority of maternal aneuploidies originate from non-disjunction during the oocyte's first meiotic division (Jacobs and Hassold, 1995), which occurs before fertilization and, therefore, does not affect pronuclear morphology.

In previous studies in IVF cycles, closely apposed pronuclei, with nucleoli aligned at the pronuclear interphase and perinuclear condensation of the cytoplasm were considered to provide good prognosis pronuclear patterns for implantation. These conclusions were drawn from results obtained after transfer on day 1, from which a pronuclear scoring system was devised (Scott and Smith, 1998). Similar results were observed in ICSI cycles, with higher pregnancy rates when day 3 embryos with good prognosis patterns were selected for transfer (Tesarik and Greco, 1999). These findings provided new criteria for selecting the best embryos for transfer, particularly when a large number of similarly good quality embryos was available for transfer at cleavage stage.

Most recent studies have based their pronuclear scoring systems solely on nucleoli distribution (Wittemer *et al*., 2000; Balaban *et al*., 2001; Fisch *et al*., 2001; Montag and Van der Ven, 2001). Yet unequal size or distance between the pronuclei have also been associated with poorer embryo quality and lower developmental rates and with a higher incidence of multinucleation (Sadowy *et al*., 1998; Scott *et al*., 2000; Zollner *et al*., 2002; Scott, 2003). In the present study, most of the zygotes showed similar PN size (group A), with only 8.1% of B type zygotes (unequal PN size). Similar results have been described by others (Scott, 2003), with 10.4% of zygotes showing Z4 patterns (unequal size or non-aligned nuclei). Furthermore, multinucleation has been correlated to higher rates of mosaicism and chromosomal abnormalities in preimplantation embryos (Kligman *et al*., 1996). In fact, in one study, a significantly higher incidence of mosaicism was found in day 3 embryos from zygotes with dysmorphic pronuclei than in those from zygotes with normal pronuclear morphology (Sadowy *et al*., 1998). Likewise, in the present study a higher incidence of chromosomal abnormalities was found in embryos originating from pronuclei with different sizes, and this value increased when the parameter of NPB distribution was included. In the present study, among patients aged ⩽37 years, there was a trend towards a higher incidence of chromosomal abnormalities in embryos from zygotes with different pronuclei sizes, and statistically significant differences were found when a second parameter—the number and distribution of NPB—was added. In other words, embryos from zygotes with equal pronuclear size and synchronized and polarized nucleoli suffered significantly less chromosomal abnormalities than embryos derived from zygotes with different PN size and asynchronic NPB. Comparisons of the second parameter (NPB distribution) as an isolated variable were impeded by the lack of zygotes with different pronuclei sizes and synchronized and polarized nucleoli.

Another parameter evaluated in PN morphology is the appearance of a cytoplasmic halo during PN formation. This is due to the contraction of organelles from the cortex to the centre of the oocyte, which leaves a clear cortical zone (Payne *et al*., 1997). Previous studies have not clearly demonstrated the effect of this parameter on embryo development (Demirel *et al*., 2001), although the presence of perinuclear condensation has been associated with higher implantation rates (Scott and Smith, 1998). Others (Balaban *et al*., 2001) found a higher percentage of good morphology embryos in halo-positive zygotes, but did not observe any statistical differences in pregnancy and implantation rates. The present data showed a significantly higher blastocyst rate in halo-positive than in halo-negative zygotes in patients aged ⩽37 years. The incidence of chromosomal abnormalities in day 3 developing embryos was slightly increased in halo-negative zygotes, although this difference was not statistically significant. This effect was not observed in patients aged >37 years.

Oocyte cytoplasmic immaturity and sperm decondensation defects could lead to the development of abnormal pronuclear patterns (Tesarik and Kopecny, 1989; Rienzi *et al*., 2002). A study performed in couples with two consecutive oocyte donation cycles showed that certain ICSI sperm samples repeatedly produced high proportions of zygotes with abnormal PN patterns. This effect was not thought to be related to any of the conventional sperm parameters; rather, the authors attributed it to a minor gene activity of the male pronucleus, to the sperm centrosome or to the sperm-derived oocyte-activating factor (Tesarik *et al*., 2002). In the present study, statistical differences were observed in the percentage of zygotes derived from normozoospermic samples between groups A and B of the younger patients, although oocyte contribution could not be completely ruled out. In a recent study, a correlation between the source of spermatozoa (ejaculate and testicle) and pronuclear patterns could not be established (Demirel *et al*., 2001). However, other authors found that the microinjection of spermatids (Tesarik and Mendoza, 1996; Kahraman *et al*., 2002) or testicular sperm into mature oocytes (Kahraman *et al*., 2002) could lead to zygotes with poor prognosis PN patterns.

The present study, and the results of other published studies, confirm that when there exists polarization of NPB in both pronuclei and the pronuclei are of similar size, embryo quality and development is improved (Sadowy *et al*., 1998; Scott and Smith, 1998; Tesarik and Greco, 1999; Scott *et al*., 2000; Balaban *et al*., 2001; De Placido *et al*., 2002; Kahraman *et al*., 2002; Rienzi *et al*., 2002; Zollner *et al*., 2002; Scott, 2003). As has already been mentioned, chromosomal abnormalities have a detrimental effect on preimplantation development. Therefore, it is not a coincidence that the PN pattern displaying a

more favourable embryo development has also been associated with a lower rate of chromosomal abnormalities (Sadowy *et al*., 1998; Kahraman *et al*., 2002). On the other hand, as also suggested by authors combining PN scoring with day 2/day 3 embryo scoring, the worst zygote patterns are the most accurate indicators of a poor IVF/ICSI outcome (De Placido *et al*., 2002).

It can be concluded that pronuclei of different size and asynchronic NPB distribution negatively affect embryo development, resulting in higher rates of chromosomal abnormalities in day 3 embryos. This embryo selection for improving IVF outcome, based on PN morphology, is useful mainly in patients aged ⩽37 years. In patients aged over 37 years, PGD for the most common aneuploidies is the best approach for limiting the risk of transmitting chromosomal abnormalities to the offspring.

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