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Correlation between embryo morphology and development and chromosomal complement

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ABSTRACT

Objective: To analyze the correlation between embryo morphology and the chromosomal status using the array comparative genomic hybridization [array comparative genomic hybridization (a-CGH)] technique for screening 23 chromosome pairs in a single blastomere biopsy from Day 3 embryos. **Methods:** One thousand five hundred and fifty seven embryos were included from 203 cycle ICSI patients undergoing preimplantation genetic screening. The 23 chromosome pairs were analyzed by blastomere biopsy from day 3 embryos using a-CGH array method. Embryo development rate, fragmentation rate and chromosome status of the analyzed blastomeres were recorded and correlated with the aCGH results. **Results:** The incidence of chromosomal abnormalities was significantly higher in slow-and fast cleaving embryos at day 3 after insemination. The incidence of fragmentation and the type of fragmentation was associated with an increased incidence of chromosomal abnormalities. The symmetry of the blastomeres also correlated with the aneuploidy rates. **Conclusions:** Embryo development rate and morphological parameter such as degree, type of fragmentation and the symmetry of the blastomeres to a large extent reflect the cytogenetic status of the embryo and thus are important in the selection of embryos with the highest implantation potential.

1. Introduction

One of the key factors for a successful outcome in an assisted reproductive program is the selection of embryos with the highest developmental potential. For many years embryos have been selected based on parameters considered important quality indicators, such as fragmentation, cell number and cell size[1-7].

The development and implementation of techniques for preimplantation genetic diagnostic programs have made it possible to assess the chromosomal constitution without destroying the embryo. It has been suggested that pronuclear morphology could be indicative of embryo quality[1,8]. Using FISH technique for pre-implantation genetic screen for 5-7 chromosomes, previous studies showed that embryos containing unevenly sized blastomeres have an increased aneuploidy rate[9,10]. Further, there is evidence that growth retardation in addition to accelerated cleavage could be an indication of chromosomal abnormalities[11-13]. Other studies have demonstrated increased chromosomal abnormality rates with increased degree of fragmentation or poor embryo morphology[11-13].

Most studies published have been based on the technique of Fluorescence In Situ Hybridization (FISH) results. This form of evaluation consists of testing 5 to 12 chromosomes from either single or dual cell biopsy in an attempt to predict the chromosomal status of the whole embryo [10,12,13]. Therefore, the information obtained is not always representative of the real chromosomal status. The adaptation of a-CGH to single cells has allowed the study of the full karyotype of blastomeres, thus identifying the true level of aneuploidy in cleavage-stage embryos, which was then reported to affect 75% of them[14-17].

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The aim of this study was to analyze the correlation between embryo morphology and the chromosomal status using the a-CGH technique for screening 23 chromosome pairs in a single blastomere biopsy from Day 3 embryos.

2. Materials and methods

2.1. Patient population, embryo culture and biopsy

203 patients undergoing *in vitro* fertilization (IVF) treatment and preimplantation genetic screening (PGS) with aCGH at Red Rock Fertility Center were included in this study. The study was conducted after obtaining the Institutional Review Board's approval. The average maternal age of patients was 34.7 years (range 29-41 years). Patients underwent one of the following orarian stimulation protocols; luteal phase Lupron suppression (Leuprolide acetate; TAP Pharmacceuticals, Lake Forest, IL) with or without oral contraceptive pretreatment; gonadotropin-releasing hormone (GnRH) antagonist prevention of premature ovulation with cetrorelix (Cetrotide; EMD Derono, Rockland, MA) or ganirelix (Organon USA, Roseland NJ). In the antagonist protocol, the GnRH antagonist was added when a lead follicles measured ≥14 mm. Controlled ovarian hyperstimulation was performed with human menopausal gonadotropin (Menopur; Ferring Pharmaceuticals, Parsippany, NJ), recombinant luteinizing hormone (LH, Luveris, EMD Serono), and/or recombinant FSH (Follistim, Organon USA; Gonal-F, EMD Serono). Cycles were monitored with serum estradiol levels and transvaginal ultrasounds. When at least 2 follicles measured ≥18 mm, 5 000-10 000 units of urinary hCG (Novarel; Ferring Pharmaceuticals) were administered subcutaneously. Ultasound-guided oocyte retrieval was performed 36 hours after hCG administration.

All mature oocytes were fertilized by intra-cytoplamic sperm injection (ICSI) method. Embryos were cultured using Global media (LifeGlobal) with 10% Serum Substitute Supllement (SSS) (Irvine Scientific) under triple gas incubator (6.5% CO_2; 5% O_2 and 88.5% N_2).

A total of 1 257 embryos were biopsied on day 3 of embryo development and underwent aneuploidy screening with aCGH. After biopsied, the embryos were culture until day 5 or day 6 of development. Euploid embryos were either tranferred to the uterus or frozen for future use.

2.2. Embryo scoring

Oocytes were checked for the presence of pronuclei and polar bodies 16-18 hours after ICSI. Fertilized oocytes were cultured and scored 66-68 hours after insemination for: cell number; degree of fragmentation (without fragmentation, less than 5%, 6% to 15 %; 16% to 30% and more than 30% fragmentation); localization of fragments (local or dispersed); equally or unequally sized blastomeres).

Biopsy procedures were carried out on day 3 (66-68 hours after insemination). One blastomere was gently aspirated with the use of a biopsy pipette. After blastomere biopsy, embryos were thoroughly rinsed and transferred to a new dish of Global media with 10% SSS and cultured to day 5 and day 6. The biopsied blastomere was transferred to the tubes and sent to a Genetics laboratory for chromosome evaluation by a-CGH. Embryos marked as euploid were chosen for transfer or frozen.

2.3. Statistical analysis

Data was analyzed by *chi*-square analysis and relative risk test (*RR* test).

3. Results

1 257 cleavage-stage embryos were biopsied and analyzed for aneuploid. A total of 783/1257 (62.3%) were aneuploidy and 474/1257 (37.7%) were euploid. All embryos were observed until the end of day 6 for developmental progress. 572 blastocysts developed from biopsied embryos, of which 257 (32.8%) were developed from aneuploidy embryos and 315 (66.5%) were developed from euploidy embryos. The competence to develop to blastocyst stage was decrease 2 times in aneuploidy embryos compare to euploidy embryos (32.8% *vs*. 66.5%); *RR* = 2; *P*<0.001 (Table 1).

Table 1
Development of biopsied embryos to blastocyst stage.

Development of D3 embryos	Aneuploid	Euploid	*RR*
Slow/ Arrested	526(67.2%)	159(33.5%)[a]	1
Blastocysts	257(32.8%)	315(66.5%)[b]	2
Total	783	474	-

a *vs*. b p <0.001

Figure 1 shows the results of 1 257 embryos which underwent tested for aneuploidy. The results were analyzed for each cellular stage in details, the lowest incidence of chromosomal abnormalities was found in embryo with 8 cells (53.9%) and the highest was found in embryos with 4-5 cells (87.2%). In human in-vitro fertilization, embryos usually develop to 8 cell stage at 54-72 hours after fertilization. In our study, we assessed the embryos at 66-68 hours after fertilization. Our results show that slow developed embryos that have 4-6 cells have significantly higher aneuploidy rate of 83.1%, nearly 1.5 times higher than embryos which have

7–9 cells (*RR*= 83.1/56.3= 1.476; *P*<0.001) and nearly 1.3 times higher than embryo which have more than 9 cells (*RR*= 83.1/65.7= 1.265; *P*<0.001). Embryos with fast development also have aneuploidy rate nearly 1.2 times higher than embryos which have 7–9 embryos (*RR*= 65.7/56.3= 1.167; *P*<0.001).

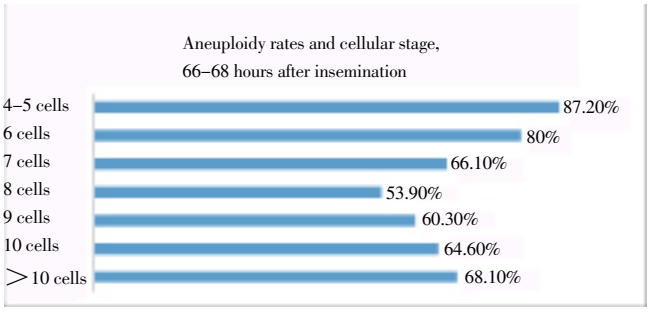

Figure 1. Chromosome abnormalities and cellular stage.

The numerical analysis of chromosomes was increased with the the percentage of fragmentation. Table 2 shows that embryos with a lot of fragmentation 16%–30% have the highest aneuploidy rate (75.1%), 1.14 times higher than embryos with moderate fragmentation 6%–15% (*RR*=75.1/65.7=1.14, *P*<0.025); 1.35 times higher than embryos with little fragmentation 1%–5% (*RR*=75.1/55.4=1.35; *P*<0.001) and 2.6 times higher than embryos without fragmentation (*RR*=75.1/28.6=2.63; *P*<0.001). The aneuploidy rate in embryos with moderate fragmentation was nearly 1.2 times higher than in embryos with little fragmentation (*RR*=65.7/55.4=1.18; *P*<0.005) and 2.3 times higher than in embryos without fragmentation (*RR*=65.7/28.6=2.29; *P*<0.001). The aneuploidy rate in embryo with little fragmentation was higher 1.9 times compared to embryo without fragmentation (*RR*=55.4/28.6=1.94; *P*<0.001) (Table 2).

Table 2

Chromosomal abnormalities and percentage of fragmentation.

Fragmentation	#tested for aneuploid	Aneuploid	Euploid	*RR*
0%	28	8(28.6%)[a]	20(71.4%)	1
1%–5%	552	306(55.4%)[b]	246(44.6%)	1.94
6%–15%	420	276(65.7%)[c]	144(34.3%)	2.29
16%–30%	257	193(75.1%)[d]	64(24.9%)	2.63
Total	1257	783	474	–

a *vs.* b *P*< 0.001; b *vs.* c *P*<0.005; c *vs.* d *P* < 0.05

As shown in Table 3, aneuploidy rate was related to the location of fragmentation. Aneuploidy rate was higher 1.9 times in embryos with fragment located scattered compare with embryo with fragment concentrated in the peripheral area (*RR*=77.6/40.1=1.94; *P*<0.001).

Table 4 shows that embryos with uneven blastomeres have 1.8 times higher aneuploidy rate compared to embryos with even blastomeres (*RR*=81.6/44.1=1.85; *P*<0.001).

Table 3

Aneuploidy rate and the location of fragment.

Location	# tested for aneuploid	Aneuploid	Euploid	*RR*
% fragmentation	28	8(28.6%)	20(71.4%)	–
Concentrated in the peripheral	476	191(40.1%)[a]	285(59.9%)	1
Scattered	753	584(77.6%)[b]	169(22.4%)	1.94
Total	1257	783	474	–

a *vs.* b *P*<0.001.

Table 4

Aneuploidy rate and the symmetry of the blastomeres.

Symmetry of the blastomeres	# tested for aneuploid	Aneuploid	Euploid	*RR*
Even blastomeres	648	286(44.1%)[a]	362(55.9%)	1
Uneven blastomeres	609	497(81.6%)[b]	112(18.4%)	1.85
Total	1257	783	474	–

a *vs.* b *P*<0.001

4. Discussion

The effectiveness of chromosomal screening methods depends on the ability to accurately distinguish euploid embryos from those affected by aneuploidy. Almost all previous pre-implantation genetic screening (PGS) studies have been based upon the use of fluorescence in situ hybridization (FISH). Although FISH has allowed accurate screening of restricted numbers of chromosomes, the method is limited in that less than one-half of the chromosomes can be enumerated in each biopsied cell. The use of a-CGH allows all of the chromosomes to be evaluated, thus revealing nearly 100% of aneuploid embryos[14,15]. Additionally, the a-CGH method provides the advantage of avoiding the technically challenging process of cell fixation on a microscope slide. The data from this study indicated that selected morphology features and embryo development rate were related to the chromosomal status of the embryo.

It has been suggested that good embryos should cleave at an optimal cleavage rate[7,18-20]. Embryos which cleave either too fast or too slow usually indicate a compromised developmental potential. In this study, embryos with a slow cleavage rate resulting in <7 cells and embryos with fast cleavage rate (>9 cells) at 68 h after fertilization showed an increased chromosomal abnormality rate from 1.5-1.2 times. In this study, we found that 62.3% of cleavage embryos were aneuploid. 66.5% of euploid embryos on day 3 were capable to develop to the blastocyst stage whereas only 32.8% of aneuploid day 3 embryo progressed to blastocyst. It is accordance with previous suggestion that culturing human embryos to the blastocyst stage instead of cleavage stage will enable the selection and identification of healthy, chromosomally normal embryos endowed with high potential for implantation[21,22]. This study helps to further clarify this well-known observation.

In our study uneven blastomeres were associated with high incidence of aneuploidy nearly 1.8 times that was accordance with previous conclusion from FISH studies that blastomere asymmetry has been linked to reduced embryo competence, reduced the implantation rate [9,10].

Finally, increasing amounts of fragmentation in the embryos at 68 h after fertilization was significantly correlated with increased chromosomal abnormality rates. This finding is in accordance with previous publications[10,11,13]. Assuming that an increased chromosomal abnormality rate is associated with a decreased implantation and pregnancy potential, this could explain the lowered implantation and pregnancy rates after transfer of fragmented embryos as found in several studies[23,24]. Ebner *et al*. found an increased malformation rate after transfer of highly fragmented embryos and the authors concluded that this might be due to a higher percentage of chromosomal disorders. In the present of scattered fragmentation, the occurrence of chromosomal abnormalities is significantly higher compared to when fragments are concentrated in one area. When fragmentation was scattered, it will affect the cell-to-cell contacts, compaction and blastocyst formation[25].

In conclusion, we found a high incidence of chromosomal abnormality in embryos from couples participating in an assisted reproductive program. Further, this study demonstrates that the embryo development rate and morphological parameters such as degree, type of fragmentation, asymmetry of the blastomeres to a large extent reflect the cytogenetic status of the embryo and thus are important in the selection of embryos with the highest implantation potential. There is still an urgent need to clarify how normal an embryo needs to be in order to be able to implant and give rise to a healthy baby. We do not know to what extent chromosomal abnormalities compromise the developmental potential of the embryo and what, if any corrective mechanisms exist within the embryo that may compensate for various degrees of chromosomal errors.

Conflict of interest statement

We declare that we have no conflict of interest.

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