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Correlation between aneuploidy and blastocyst quality

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ABSTRACT

Objective: To carry out a detailed investigation of the impact of aneuploidy on blastocyst quality. **Methods:** 1 257 cleavage-stage embryos from 203 patients underwent IVF treatment with aneuploidy screening were investigated. Comprehensive chromosome analysis involved the use of microarray comparative genomic hybridisation (aCGH) method on single blastomere biopsied on day 3 of embryo development. Embryo morphology was assessed and recorded on days 3 and/or 5-6 post-fertilisation. Morphologic grade A-C was assigned to the blastocysts with grade A being the highest grade and grade C the lowest. **Results:** Among the 1 257 embryos that were analyzed, 474 (38%) were euploid, and 783 (62%) were aneuploid. 315/474 (66.5%) of euploid embryos developed into blastocysts, while 57/783 (32.8%) of aneuploid embryos grew to blastocysts ($P<0.01$). At the blastocyst stage, the best quality embryos (grade A) were in their majority (164/241 or 68%) euploid. Of the 219 fair quality blastocyts (grade B) 129 were euploid (59%) and 22/112 (20%) of poor quality blastocysts (grade C) were euploid. **Conclusion:** Blastocyst morphology showed a significant link to aneuploidy ($P<0.05$). However, grading morphology alone can not replace preimplantation aneuploidy screening. Morphology screening or other markers for embryo competence, combined with preimplantation genetic screening for 24 chromosomes may produce the best results.

1. Introduction

Chromosomal abnormalities in human oocytes and pre-implantation embryos are extremely common and increase significantly with advancing maternal age[1]. It is believed that in most cases aneuploidy embryos either arrest prior to transfer to the mother, fail to implant after transfer, or spontaneously abort early in gestation. To maximize the success rates of *in vitro* fertilization (IVF) treatments, a reliable means of recognizing the embryo with the greatest potential for producing a pregnancy is required. Currently, most methods of embryo evaluation involve the assessment of morphologic criteria, such as cell size and number, multinucleation, and the percentage of the embryo volume occupied by cellular fragments[2]. However, it is acknowledged that such characteristics are only weakly correlated with IVF outcome, and consequently morphologic grading provides only a limited guide to embryo viability. Embryos achieving the best morphologic scores often fail to achieve implantation or do not produce a live birth, and those with poor scores sometimes succeed in producing a child. In many cases, the underlying cause of embryo arrest, implantation failure, or spontaneous pregnancy loss is the presence of numerical chromosomal abnormalities (aneuploidy). Most of the information concerning the chromosome complement of human pre-implantation embryos comes from the examination of the cleavage stage of development, 3 days after fertilization. Data has been obtained during the course of pre-implantation genetic screening (PGS) cycles, in which single cells biopsied from cleavage stage embryos are analyzed using fluorescent in situ hybridization (FISH)[3-4]. Data from multiple FISH

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studies, carried out in many laboratories around the world, have confirmed the high frequency of aneuploidy in early human embryos.

In the vast majority of cases, a chromosomal imbalance of this type is lethal to the developing embryo. Many studies have searched for a link between aneuploidy and altered embryo morphology[5-6]. Some associations have been identified, e.g., aneuploidy rates are increased in embryos exhibiting cellular fragmentation, unevenly sized cells, multinucleation, or atypical cell numbers. However, in most cases, these correlations are weak. It is possible that the failure to find a reliable morphologic indicator of aneuploidy in previous studies has been a consequence of technical limitations. Also, cleavage stage embryos have higher levels of aneuploidy than blastocyst stage embryos[7]. Several studies have shown that blastocysts transferred on day 5 had higher implantation and pregnancy rates than those transferred on day 6, suggesting improved viablility for faster developing embryos[8-9]. Virtually all research and clinical investigations have assessed only a limited number of chromosomes in each embryo, and it is therefore inevitable that some of the embryos categorized as euploid were in fact abnormal, with aneuploidies affecting chromosomes that were not tested. Therefore, we aimed to carry out a more detailed inverstigation of the impact of aneuploidy on blastocyst formation and morphology. For this purpose the copy numbers of all 24 types of chromosomes were evaluated.

2. Materials and methods

2.1. Patient population, embryo culture and biopsy

203 patients undergoing IVF and 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 ICSI method. Embryos were cultured using Global media (LifeGlobal) with 10% 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

Embryos were assessed for their stage of develoment and morphologic grade on days 1, 3, 5 and 6. Embryos reaching the blastocyst stage were graded using the Gardner system [10]. Blastocysts were assigned a number based on the degree of expansion and hatching status (from 1 to 6): 1= early blastocyst: blastocele accounts for less than one-half of the volume of the embryos; 2=blastocyst: the blastocele occupies more than one-half of the volume of the embryo; 3=full blastocyst: the blastocele fills the embryo completely; 4=expanded blastocyst: the blastocoele is now larger than the early embryo and the zona pellucida has begun to thin; 5=hatching blastocyst: trophectoderm (TE) cells have begun to herniate through the zona pellucida and 6= hatched blastocyst: the blastocyst has completely escaped the zona pellucida.

A second scoring step was performed under an inverted microscope to assess the inner cell mass (ICM) and the TE. For the ICM, the following descriptions are used: A=tightly packed with many cells; B=loosely grouped with several cells; and C=very few cells. For the TE, the following grading is used: A=many cells forming a cohesive epithelium; B=few cells forming a loose epithelium; and C=very few large cells. Blastocysts were considered "good" when containing AA, AB or BA; "fair" when containing BB and "poor" when containing BC, CB or CC.

1 257 cleavage-stage embryos from 203 patients (average maternal age 34.7 years ±5.5) were investigated. Comprehensive chromosome analysis involved the

use of microarray comparative genomic hybridisation (aCGH) method on single blastomere biopsied on day 3 of embryo development. Embryo morphology was assessed and recorded on days 3 and/or 5–6 post-fertilisation. Morphologic grade A–C was assigned to the blastocysts with grade A being the highest grade and grade C the lowest.2.3. Statistical analysis

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

3. Results

Among the 1 257 embryos that were analyzed for aneuploidy, 474 (38%) were euploid and 783 (62%) were aneuploid (Figure 1).

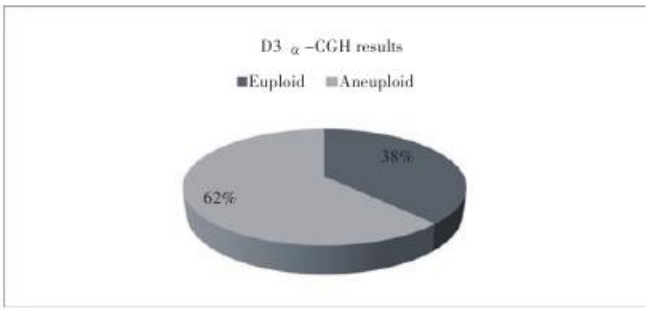

Figure 1. D3 α-CGH results.

Table 1

The development of D3 biopsied embryos to blastocyst stage (*n*, %).

Development of D3 embryos	Aneuploid	Euploid
Slow/Arrested embryos	526 (67.2)	159 (33.5)
Blastocysts	257 (32.8)	315 (66.5)*
Total	783(100.0))	474(100.0)

*P<0.01when compared with aneuploidy embryos grew to blastocysts.

As shown in Table 1, 315/474 (66.5%) of euploid embyos developed into blastocysts while 257/783 (32.8%) of aneuploid embryos grew to blastocysts (P<0.01).

Of the 1 257 day 3 embryos that were chromosomally analyzed using aCGH, 39.9% were developed into early blastocysts on day 5, 26.9% were developed blastocysts with clearly seen ICM and TE. Aneuploidy rates increased nearly 3.4 times in arrested and morular embryos compared to good quality blastocysts. (*RR* = 3.43 and 3.37, P<0.01). Of the 338 embryos developed to full blastocysts (blastocysts grade A, B and C) atleast on day 5, 29.9% (101/338) were aneuploid and 70.1% (237/338) were euploid. The aneuploidy rate of early blastocysts was higher 2.4 times higher compared to fully developed blastocysts (*RR*=2.41, P<0.01). On day 5, the aneuploidy rates were correlated with the quality of the blastocysts, the rate was highest in poor embryos and lowest in good embryos, 2.5 times higher compared to good blastocysts (*RR*=2.52, P<0.01). There was no difference between the aneuploidy rates of good and fair blastocysts (Table 2)

Table 2

Blastocyst formation and morphology on day 5.

Development	Euploid	Aneuploid	Total	*RR*
Arrested	34 (11.1%)	273 (88.9%)**	307	3.43
Morular	14 (12.7%)	96 (87.3%)*	110	3.37
Cavitated (early blastocyst)	189 (37.6%)	313 (62.4%)#	502	2.41
Grade A blastocyst	146 (74.1%)	51 (25.9%)▽	197	1
Grade B blastocyst	83 (70.3%)	35 (29.7%)▽	118	1.15
Grade C blastocyst	8 (34.8%)	15 (65.2%)	23	2.52
Total	474	783	1 257	

*P<0.05 when compared with aneuploidy rates of cavitated (early blastocysts) and blatstocysts (grade A, grade B and grade C); **P<0.01 when compared with aneuploidy rates of cavitated (early blastocysts) and blatstocysts (grade A, grade B and grade C); #P<0.01 when compared with aneuploidy rates of grade A and grade B blatstocysts; ▽P<0.01 when compared with aneuploidy rates of grade C blastocysts.

As in day 5 blastocysts, blastocysts formed on day 6 with good and fair quality had the lowest aneuploidy rates compared to slow, arrested embryos and poor blastocysts (P<0.01). Slow, arrested embryos and poor quality blastocysts had the aneuploidy rates increased by 1.6 times compared to good and fair blastocysts (Table 3).

Table 3

Blastocyst formation and morphology on day 6.

Development	Euploid	Aneuploid	Total	RR
Slow arrested at morular stage or blastocyst stage	34 (11.6%)	258 (88.4%)*	292	1.6
Grade A blastocyst	18 (40.9%)	26 (59.1%)▽	44	1.1
Grade B blastocyst	46 (45.5%)	55 (54.5%)▽	101	1
Grade C blastocyst	14 (15.7%)	75 (84.3%)	89	1.55
Total	112	414	526	

*P<0.01 when compared with aneuploidy rates of grade A and B blastocysts; ▽P<0.01 when compared with aneuploidy rates of grade C blatstocysts.

At the blastocyst stage the best quality embryos (grade A) were in their majority (164/241 or 68%%) euploid. Of the 219 average quality blastocyts (grade B) 129 were euploid (59%) and 22/112 (20%) of poor quality blastocysts (grade C) were euploid. There is significant increase in the aneuploidy rates of poor blastocysts compared to good and fair quality blastocysts (P<0.01) (Figure 2).

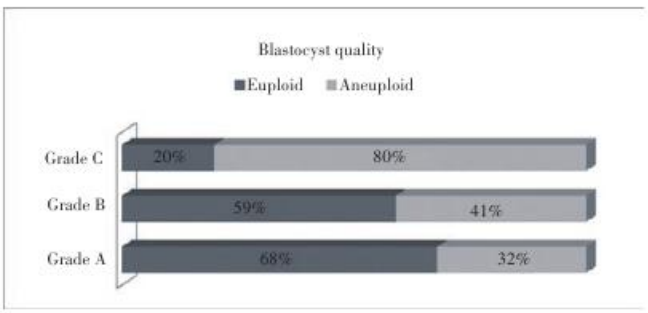

Figure 2. Blastocyst quality and aneuploidy rate.

4. Discussion

Many studies have searched for a link between aneuploidy and altered embryo morphology[5-6, 11]. Some associations have been identified, e.g., aneuploidy rates are increased in embryo exhibiting cellular fragmentation, uevenly sized cells, multinucleation, or atypical cell numbers. However, in most cases there correlation are weak[12-13]. It is possible that the failure to find a reliable morphologic indicator of aneuploidy in previous studies has been the consequence of technical limitations. Most of those studies used FISH for chromosomal analysis and did not examine the association between the rate of embryo development and aneuploidy. Despite the apparent association between day 3 morphology and chromosomal content, a significant proportion of morphologically high quality day 3 embryos are aneuploid[14]. This finding may be accounted for the fact that the embryonic genome is not activated until the four to eight cell stage. Up until day 3 after fertilitzation, the survival of embryos is facillitated by the persistence of proteins derived from the oocyte. However, after the onset of embryonic gene expression at the 4-8 cell stage the embryo must increasingly rely on the product of its own genome. Thus aneuploidy is likely to have a progressively more dramatic effect as development advance beyond day 3. By extending culture to the blastocyst stage, exclusion of aneuploid embryos that experience arrested development on day 2 or 3 may therefore be possible[15]. Qi *et al* demonstrated that aneuploid embryos are significantly more likely than euploid embryos to experience arrested development between day 3 and day 5[16]. Moreover, there is increasing evidence for improved implantation and pregnancy rates when culture is extended to day 5. It has been postulated that culture to the blastocyst stage aids in the elimination of chromosomally abnormal embryos. Our data confirmed that some aneuploid embryos are eliminated before the blastocyst stage and culture to the blastocyst stage helps to select for euploid embryos but not definitively. 66.5% of euploid embryos will developed to blastocyst stage, while only 32.8% aneuploid embryos can growth to blastocysts (P<0.001).

More recently, a few studies have further characterized embryo aneuploidy with the use of aCGH, although they have focused on high-morphologic quality blastocysts without assessing embryo developmental progression[17]. In the present study using aCGH, aneuploidy rates were correlated with embryo morphology and development. The use of aCGH was intended to identify the 19%-58% of chromosomal abnormalities that would otherwise have been missed with FISH analysis[17] and embryo development and morphology on day 5 and day 6 was assessed because of the common clinical practice of selecting blastocysts for transfer at that time. In our study, of the 1 257 embryos studied, 45.5% of those reaching blastocyst stage by day five or day six. 44.9% of the blastocysts were aneuploid. This aneuploidy rate is in comparable with other studies using aCGH which have reported overall aneuploidy rates ranging from 38.8% to 56.7%[12, 17-18]. Many of the highly abnormal embryos detected during this present stuty had poor TE and ICM. Regarding blastocyst morphology as an indicator of chromosome abnormality, we found increased aneuploidy among blasotcyst with poor morphologic scores and a greater likelihood of euploidy for embryos with good scores (68% euploid for grade A and 59% euploid for grade B). and only 20% of poor quality blastocysts were euploid.

On day 5, we showed that cavitated embryos (early blasotocyst) and morula, arrested embryos had 2.4 to 3.4 times higher aneuploidy rates than good and fair quality blastocysts. The aneuploidy rates was lower in cavitated embryos (early blastocysts) than in morula embryos on day 5. There was no significant difference between aneuploidy rates in good and fair quality embryos. However, when the quality of the embryos drops to poor morphology grade C the aneuploidy rate was increased significantly by 2.5 times compared to good and fair quality blastocysts (grade A and B). Our finding was the same with previous study of Kroener *et al* [19] said that when assessing morphology independently from developmental stage, an association was noted between aneuploidy and morphologic quality and this association was significant only when embryo morphology dropped to grade C (poor quality). Therefore, morphologic grade and developmental stage may be useful for selecting normal embryos.

On day 6, same as on day 5 cavitated, morula, arrested embryos had 1.6 times higher aneuploidy rates than good and fair quality blastocysts. Poor quality blastocysts has 1.5 times higher aneuploidy rates compared to better blastocysts.

In summary, aneuploidy affected nearly two-third of the cleavage embryos in this study. Euploidy embryos have more chance to developed to the blastocyst stage than aneuploidy embryos (66.5% *vs.* 32.6%). Of all the blastocysts formed on day 5 and 6, euploidy embryos accounted for 55.5%. Aneuploidy were shown to be correlated with blastocyst formation and morphology. The slower development and the poorer morphology are associated

with higher aneuploidy rates. Blastocyst morphology and aneuploid are linked. However, morphologic analysis cannot be relied on to ensure the transfer of chromosomally normal embryos. A significant proportion of aneuploid embryos are capable of achieving the good morphologic scores, certain abnormalities are compatible with development to the blastocyst stage, implantation, and subsequent development to term, and some euploid embryos are morphologically poor. At the present time, PGS still remains the only effective means to detect euploid embryos for transfer. The optimal way to select euploid embryos is through a combination of morphology grading and PGS screening for all 24 chromosomes.

Conflict of interest statement

We declare that we have no conflict of interest.

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