Original Article

Embryo Fragmentation and its Relationship with Aneuploidy

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Abstract

Objective: Selecting embryos is an important step in the *in vitro* fertilization process before transferring them to the uterus. There are some invasive methods for choosing a good quality embryo, such as embryo grading. This method evaluates the equality and fragmentation of an embryo. However, this method does not adequately evaluate the chromosomal status of the embryos, which is often necessary for high-risk embryos. Here, we evaluated embryo fragmentation and chromosomal numbers using next-generation sequencing. **Materials and Methods:** Each embryo was biopsied on the 3rd or 5th culture day to obtain a single blastomere cell. DNA was then extracted from each blastomere and whole-genome amplification was carried out. Amplification products were then sequenced to obtain a ploidy number. **Results:** Among the 30 embryos that were evaluated, 19 embryos had no fragments, 10 embryos had small fragments, and 1 embryo had moderate fragments. However, 12 of 19 embryos, 57.9% with no fragments were detected to have chromosomal abnormality. Aneuploidy was increased in 7 of 10 embryos (70%) with mild fragments. One moderately fragmented embryo included was surprisingly found to have normal ploidy (100%). Gamma correlation test showed that there was no correlation between fragmentation and the incidence rate of aneuploidy ($P > 0.05$). Although there was no correlation, the study's result exemplifies that aneuploidy rate increased along with higher fragmentation. **Conclusion:** This research concluded that embryo fragmentation was not correlated with aneuploidy.

Keywords: Aneuploidy, embryo, euploid, fragments

Introduction

Selecting embryos is an important step in the *in vitro* fertilization (IVF) process. This selection process takes place before transferring the embryo to the uterus. Transfers carried out with good quality embryos increase the success rate of IVF. Poor quality embryos pose many risks if transferred, such as miscarriage, low rate of embryo implantation, and babies born with genetic birth defects.[1,2] Therefore, it is necessary to use precise selection methods to ensure good quality embryos are selected. There are some methods to invasively choose a good quality embryo, such as embryo grading, by evaluating embryo morphology.[3] Embryos were categorized by the level of quality to ensure only the best were chosen for transfer to the uterus. One widely used method of embryo grading is the Veeck criteria.[4] These criteria classify embryos into five levels based on equality, number of cells, and fragmentation. Level 1 shows the best quality embryos, while level 5 shows the lowest quality embryos. Previous researches showed that embryos with low grading have a higher risk of disorders, such as chromosomal abnormalities and other genetic disorders. This cross-sectional study focused on finding the relationship between fragmentation, one of the most assessed morphological characteristics, and the incidence of aneuploidy.

Embryo fragmentation occurs when nuclear chromosomes are cutoff into smaller parts. The fragmented DNA then leaves the cell and forms blebs. The amount of fragmentation experienced by an embryo is assessed based on the number of small blebs formed outside of the cells. Veeck (1988) classified embryo fragmentation into the following four groups: embryos without fragmentation, mild fragmentation,

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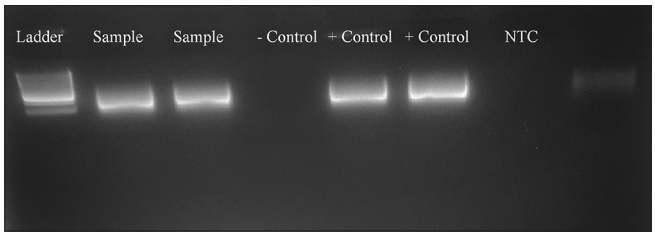

Figure 1: Gel electrophoresis result

moderate fragmentation, and excess fragmentation.[3] Fragmentation is associated with poor embryo quality caused by a high rate of aneuploidy.[2,5] Aneuploidy is a chromosomal number abnormality characterized by increasing or decreasing the number of chromosomes in a pair. A chromosome pair that loses one chromosome is called monosomy, whereas a chromosome pair that gains extra chromosomes is called trisomy.[6] In general, the occurrence of aneuploidy is caused by a failure in separating chromosomes during meiotic division.[7] In embryos, the occurrence of aneuploidy is caused by several factors, such as chromosome nondisjunction, premature separation of homologous chromatids, segregation defects in oocyte meiosis, cellular stress, and mosaic gonadal cells.[8,9] Altered chromosome combination and advancing maternal age are both considered as the cause of abnormal chromosome numbers, in spite of their unclear mechanism. Embryos that have poor morphological characteristics may still have a normal genetic status and the potential to be born free of birth defects. However, further evaluation is needed, especially for high-risk embryos.[4] Preimplantation genetic screening (PGS) is one method of examining the chromosomal statuses of embryos. PGS has a success rate above 90%, which is considered reliable and valid.

The aim of PGS is to choose the embryo with the highest potential to achieve most stable pregnancy.[10] PGS was done basically to obtain chromosomal status of the embryos. There are several methods to perform PGS, such as fluorescence *in situ* hybridization, array comparative genomic hybridization (aCGH), quantitative real-time polymerase chain reaction, BoBs Karyolite platform by Luminex, and next-generation sequencing (NGS).[11,12] aCGH methods performed by comparing sample chromosomes with control through hybridization method. Meanwhile, NGS was done by sequencing each chromosome of the cell and took the shorter time of running and hands-on process. The success rate of IVF with PGS increases when the NGS method is chosen rather than aCGH (71.6% vs. 64.6%).[13] Therefore, PGS through NGS analysis is a widely used method to check the embryo's chromosome status. This research was conducted to determine whether there was a correlation between embryo selections based on morphology, focusing on the level of fragmentation, and aneuploidy status evaluated by PGS. We tested the hypothesis that there was a positive correlation between embryo fragmentation and the aneuploidy status of embryos.

Materials and Methods

This was a cross-sectional study where 30 embryos were analyzed according to the inclusion criteria. Embryos were obtained from Yasmin IVF Clinic, Cipto Mangunkusumo General Hospital, Jakarta, from patients who underwent the IVF process. This study passed the ethical review and has been approved by the Ethics Committee of the Faculty of Medicine, University of Indonesia, under protocol number 17-09-0877. The embryos morphological characteristics were assessed on the 3rd day of culture. The parameters assessed were fragmentation, equivalency, and cell number. Each embryo was then graded based on the Veeck criteria.

The embryos were biopsied to obtain 1–3 blastomere on the 3rd or 5th cultured day. Biopsies were performed on each embryo by piercing the zona pellucida under the microscope with a laser (Octax). DNA from each embryo was then extracted and amplified through the Whole Genome Amplification process using a 24 sure Plex Kit (Illumina). The amplification product was quantified using Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific) and quality checked using gel electrophoresis [Figure 1]. Quantified whole-genome from embryos went through the process of labeling, hybridization, neutralization, and pooling based on PGS V3 kit procedures by Illumina and sequenced using Mi-Seq instruments (Illumina). Sequencing result were analyzed using BlueFuse Multi Software by Illumina to gain each chromosomal (autosomal and gonosomal) number.

Statistical analysis was performed using a gamma test. This test aimed to evaluate the hypothesis of ordinal correlative variables. Variables in the study would be considered to have a correlation if the *P* value obtained was < 0.05.

Results

A total of 30 embryos were successfully analyzed in this study. The grading of these embryos was conducted by assessing the embryo's equality, cell number, and fragmentation based on Veeck criteria. Based on this grading, 16 embryos were classified as Grade 1, 12 embryos were classified as Grade 2, and 2 embryos were classified as Grade 3. There were no Grade 4 or 5 embryos found in this study. Embryo fragmentation was categorized into the following four groups: no fragmentation, mild fragmentation (<10%), moderate fragmentation (<25%), and severe fragmentation (>25%). Of the 30 embryos studied, 19 had no fragmentation, 10 had mild fragmentation (<10%), and 1 had moderate fragmentation (<25%). No embryos were found to have severe fragmentation [Table 1].

Examination of the chromosomal number (ploidy) was conducted using the NGS method and Illumina Mi-Seq instruments. A total of 12 embryos had a normal amount of ploidy (euploidy). This means that each of the 23 chromosomes consisted of 2 homologous chromosomes. The other 18 embryos experienced abnormalities of ploidy (aneuploidy). Of these 18 embryos, 4 experienced monosomy, 1 experienced trisomy, and

the remaining embryos were found to have both monosomy and trisomy. Based on fragmentation, 11 out of 19 embryos without fragmentation experienced aneuploidy (58.3%), while the rest were normal. The percentage of aneuploidy increased in embryos with mild fragmentation, which is 70% or as many as 7 of 10 embryos. Meanwhile, 100% of embryos with fragmentation were found to have normal chromosome numbers (only 1 embryo in this study) [Table 2]. No correlation was observed between the fragmentation level of embryos and their ploidy numbers.

Discussion

The results showed that the higher the level of embryo fragmentation, the greater the percentage of aneuploidy incidence. In the embryo group without fragmentation, the percentage of aneuploidy was 57.9%. This number was increased to 70% in embryos with mild fragmentation. In addition, this study showed that embryos with a good morphology did not necessarily have a good chromosome status. Nineteen embryos did not have fragmentation and were grouped into Level 1. However, the sequencing results of each chromosome showed that 11 of 19 morphologically good embryos turned out to have abnormal chromosomes. The opposite result was obtained in 3 of the 10 embryos that had mild fragmentation and turned out to have a normal chromosome number. Furthermore, the only embryo that had moderate fragmentation did not show aneuploidy. The embryo had a normal chromosome number based on the sequencing of each chromosome result. This result strengthened the idea that morphological examination results do not necessarily describe the chromosomal and genetic status of an embryo. According to Gianaroli *et al.*, the morphological examination and selection method still results in errors of approximately 30%.[14] This finding corresponded with our result that as much as 9 Grade 1 embryos, which should be the best quality embryo, still possess abnormality in their chromosomes status.

To obtain more definite results, a more invasive method is required, such as whole-genome sequencing.[15,16] This sequencing is conducted as a method for screening embryos before their transfer to the uterus and aims to precisely determine the amount of chromosomes possessed by the embryo. This method has a 95% confidence level; therefore, it is believed to be an effective method for assessing embryo chromosome status.[16] The use of this method is expected to provide a more accurate picture of embryo quality so that it can increase the success rate of IVF. No correlation was observed between fragmentation and aneuploidy in embryos. However, the results of the research showed that the incidence of aneuploidy increases in embryos along with more fragmentation occurred. This suggests that fragmentation evaluation itself is not precise enough to examine the chromosomal status of an embryo.

Table 1: Embryo fragmentation

Embryo fragmentation	Total	Aneuploidy (%)	Euploidy (%)	Correlation coefficient (*r*)	*P*
No fragment	19	11 (57.9)	8 (42.1)	−0.047	0.900
Small fragments	10	7 (70)	3 (30)		
Fragments	1	0	1 (100)		
Excess fragments	0	0	0		
Total	30	18	12		

Table 2: Embryo grading result

Embryo grade	Total	Aneuploidy (%)	Euploidy (%)	Correlation coefficient (*r*)	*P*
1	16	9 (56.3)	7 (43.8)	−0.237	0.479
2	12	7 (58.3)	5 (41.7)		
3	2	2 (100)	0		
4	0	0	0		
5	0	0	0		
Total	30				

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Conflicts of interest

There are no conflicts of interest.

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