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Late embryo cleavage as an indicator of chromosome aneuploidy by pre-implantation genetic screening

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Abstract. During in vitro fertilization (IVF), not all patients receive good quality oocytes or embryos. This study investigated the frequency of aneuploidy and mosaicism in embryos obtained from parthenogenetic oocytes and poor morphological embryos that also exhibited late cleavage development by preimplantation genetic screening (PGS). Twenty late cleavage embryos under IVF treatment were cultured until day 5, and one of the blastomeres was biopsied. The embryos were divided into three groups (parthenogenetic oocytes and poor-quality embryos with dark cytoplasm, several vacuoles or fragments in the cytoplasm; arrested embryos; and good-quality embryos). All samples were tested for abnormalities using array-comparative genomic hybridization (a-CGH). Twenty late cleavage-stage embryos were biopsied and analyzed for aneuploidy. A total 17 of 20 (85%) embryos were aneuploid and three (15%) were euploid. The incidence of aneuploidy in good-quality embryos was 75%, while poor-quality embryos displayed a higher frequency (86.7%) of aneuploidy. The parthenogenetic oocytes were invariably aneuploid (100%). Based on the embryo-grading system by Veeck, embryo grades 1 and 2 exhibited a lower incidence of aneuploidy (75%), while grades 3 and 4 showed 1.33-times higher aneuploidy rates (RR = 1.33; p < 0.001). The numerical defects of the chromosomes increased with the percent of fragmentation, with fragmented embryos showing 1.32-times greater aneuploidy rates than the non-fragmented ones. Embryos with uneven blastomeres showed 1.07-times higher aneuploidy rate when compared with embryos showing even blastomeres (p < 0.0064). Late embryo cleavage results in a higher incidence of aneuploidy and mosaicism upon comparison with early embryo cleavage.
1. Introduction
In assisted reproduction, not all patients receive a good embryo or oocyte. In fact, the oocyte and sperm quality affect the development of an embryo and hence the outcome of the pregnancy. Approximately 3% of all embryos cultured in vitro are parthenogenetic oocytes that may initiate early embryonic development, leading to pre-implantation embryonic death, implantation failure, and spontaneous abortions. Embryo quality is correlated to the oocyte and zygote morphology, for example, the appearance of the cytoplasm, pro-nuclei, and polar bodies [1].

Morphological assessment is expected to assess the quality of the embryo used for in vitro fertilization (IVF). Research suggests that deterioration in embryo quality affects its morphology and the success rate of the pregnancy. Furthermore, chromosomal abnormalities such as aneuploidy occur in the embryo. Abnormal embryonic development as early as on day 2 after intracytoplasmic sperm injection with >40% fragmentation or dark cytoplasm leads to recurrent IVF failure. Ziebe et al [2], reported that the morphology is linked to the chromosomal status of an embryo. Moreover, the incidence of abnormalities has been reported to be significantly higher in slow- and fast-cleaving embryos at day 3 post-insemination. The occurrence of fragmentation and its type have been associated with an increased prevalence of chromosomal abnormalities. The symmetry of the blastomeres has also been linked with the aneuploidy rates [3].

In the present study, we investigated the frequency of aneuploidy and mosaicism in embryos obtained from parthenogenetic oocytes and embryos with poor morphology using preimplantation genetic screening (PGS).

2. Methods
The study protocol was approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Indonesia-Cipto Mangunkusumo Hospital. In this study, embryos under IVF treatment that were found to be abnormal or morphologically insufficient were cultured until day 5. Subsequently, one of the blastomeres was biopsied at YasmiN Clinic, Jakarta, Indonesia from June 2014 to June 2016.

A total of 22 embryos were divided into three groups: parthenogenetic oocytes and poor-quality embryos with dark cytoplasm, several vacuoles, or fragments in the cytoplasm; arrested embryos; and good-quality embryos. All samples were checked for abnormalities using array-comparative genomic hybridization (a-CGH). Biopsy was performed by piercing the zonapellucida with a laser (Octax) under the microscope. Next, whole genome amplification (WGA) was conducted by the WGA PicoPLEX assay. The quality of the DNA was tested using gel electrophoresis, and the product was stored at -20°C. Labeling, purification, and hybridization were accomplished using the KaryoLite BoBs assay.

The entire patient data and the test results were recorded in a log book. The percent of pregnancies that occurred in the study group was calculated. The success rate of screening procedures for genetic disorders in Indonesia was thereby obtained.

3. Results
The 22 cleavage-staged embryos were biopsied and analyzed for aneuploidy. A total of 17 out of 20 (85%) embryos were aneuploid and three (15%) were euploid. All embryos were observed until the end of day 5 for morphological and developmental progress. The numerical defects of the chromosomes were found to be proportionate to the percent of fragmentation.
Table 1. Embryo quality based on morphology

| Embryo Quality | Total | Aneuploidy | Euploidy | RR | p value |
|----------------|-------|------------|----------|----|---------|
| Poor           | 15    | 13 (86.7%) | 2 (13.3%)| 1  |         |
| Good           | 4     | 3 (75%)    | 1 (25%)  | 0.87| 0.576   |
| Parthenogenesis| 2     | 1 (100%)   | 0 (0%)   | 1.15| 1.000   |
| Total          | 20    | 17         | 3        |     |         |

Table 1 portrays the results of the 20 embryos that were tested for aneuploidy. The lowest incidence of chromosomal abnormalities was found in good-quality embryos (75%), the poor-quality ones exhibited a higher incidence (86.7%) of abnormalities, while the parthenogenetic oocytes were invariably aneuploid (100%).

Table 2. Aneuploidy rate and embryo grading

| Embryo Quality | Total | Aneuploidy | Euploidy | RR | p value |
|----------------|-------|------------|----------|----|---------|
| Grading 1      | 4     | 3 (75%)    | 1 (25%)  | 1  |         |
| Grading 2      | 8     | 6 (75%)    | 2 (25%)  | 1  | 1.000   |
| Grading 3      | 4     | 4 (100%)   | 0 (0%)   | 1.33| 0.999   |
| Grading 4      | 3     | 3 (100%)   | 0 (0%)   | 1.33| 0.999   |
| Total          | 19    | 16         | 3        |    |         |

Embryo grading was performed according to the Veeck Criteria and based on the symmetry of the blastomeres and fragmentation of the cytoplasm (Table 2). Embryo grades 1 and 2 displayed a lower incidence of aneuploidy (75%), while those with grades 3 and 4 presented 1.33-times higher aneuploidy rates (RR = 1.33; P < 0.001).

Table 3. Chromosomal abnormalities and percentage of fragmentation

| Fragments       | Total | Aneuploidy | Euploidy | RR | p value |
|-----------------|-------|------------|----------|----|---------|
| No fragment     | 11    | 9 (81.8%)  | 2 (18.2%)| 1  |         |
| Small fragment  | 3     | 2 (66.7%)  | 1 (33.3%)| 0.82| 0.577   |
| Fragment        | 2     | 2 (100%)   | 0 (0%)   | 1.22| 0.999   |
| Excess fragment | 2     | 2 (100%)   | 0 (0%)   | 1.22| 0.999   |
| Total           | 18    | 15         | 3        |    |         |

The numerical defects of the chromosomes increased with the percent of fragmentation. Table 3 reveals that embryos with a lot of fragmentation (fragments and excess fragments) exhibited the highest aneuploidy rates (100%), which is 1.32-times greater than those with small fragmentation (66.7%). The rate was also related to the blastomere symmetry.
Table 4. Aneuploidy rate and the symmetry of the blastomeres

| Symmetry | Total | Aneuploidy | Euploidy | RR  |
|----------|-------|------------|----------|-----|
| Even     | 11    | 9 (81.8%)  | 2 (18.2%)| 1   |
| Uneven   | 8     | 7 (87.5%)  | 1 (12.5%)| 1.07|
| Total    | 19    | 16         | 3        |     |

4. Discussion
Our research thus established that aneuploidy is related to a high percent of fragmentation and symmetry of the blastomeres. Our findings are in agreement with those of previous publication by Munne [4], which states that blastomere asymmetry is linked to reduced embryo competence and implantation rate. Moreover, similar results have been reported by VyPhan et al [5], who documented that the embryo development rate and morphological parameters such as degree, type of fragmentation, and symmetry of the blastomeres reflect the cytogenetic status of an embryo to a large extent and are thus important for consideration in the selection of embryos with the highest implantation potential.

This study found that higher fragmentation in the embryos was significantly correlated with increased rates of chromosomal abnormalities. This observation is in accordance with that of another research that also testified the association of chromosomal abnormality with decreased implantation and pregnancy potential. Together, these results explain the lowered implantation and pregnancy rates after transfer of fragmented embryos, as unearthed in several other studies. For instance, Ebner et al [1] observed an increased malformation rate after the transfer of highly fragmented embryos, which may be attributed to the higher percent of chromosomal disorders. VyPhan stated that blastocyst morphology was substantially linked to aneuploidy (p < 0.05). However, morphology grading alone cannot replace preimplantation aneuploidy screening [3,5].

5. Conclusion
This work thus reveals that parthenogenetic oocytes and poor-quality embryos possess a higher incidence of aneuploidy and mosaicism as compared with good-quality embryos. The percent of fragmentation and symmetry of the blastomeres was found to be related to the rate of aneuploidy. Our results corroborate that the embryo development rate and morphological parameters such as degree and type of fragmentation as well as symmetry of the blastomeres reflect the cytogenetic status of an embryo to a great extent. Thus, these factors should be considered in the selection of embryos with the highest implantation potential.

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