Title: Analysis of aneuploidies in blastocysts of multinucleated blastomeres using the Preimplantation Genetic Test (PGT-A)

Objective: The objective of this study was to analyze numerical chromosomal alterations in blastocysts using Preimplantation Genetic Testing for Aneuploidy (PGT-A), correlating them with the presence of multinucleation, degree of fragmentation, maternal age, and subsequent pregnancy after the transfer of a euploid embryo.

Methods: A retrospective study was conducted at Reproferty clinic involving patients who underwent infertility treatment using intracytoplasmic sperm injection (ICSI), followed by embryonic biopsy and Preimplantation Genetic Testing for Aneuploidy (PGT-A), from March 2021 to March 2023. Data were collected regarding patient name, age, biopsy date, identification of analyzed embryos, and PGT-A results. Time-lapse technology was used to assess potential morphological alterations such as multinucleation and degree of fragmentation, based on retrospective images from the EmbryoViewer software, integrated with the EmbryoScope incubator. All embryos identified in the PGT-A reports were checked and classified based on nuclear morphology (One nucleus, Binucleation, Multinucleation) and degree of fragmentation (Grade I: fragmentation < 10%; Grade II: fragmentation 10-25%; Grade III: fragmentation 25-50%; Grade IV: fragmentation > 50%). The clinical history was used to determine whether embryo transfer was performed or not, classifying the outcomes as: no transfer, no euploid embryos, positive Beta hCG, negative Beta hCG, biochemical pregnancy, ectopic pregnancy, and miscarriage. After data collection, percentages were calculated, and statistical analysis was performed. The groups were analyzed using the Chi-square test, considering a significance level of 99% (p<0.01), and correlation analysis was conducted.

Results: During the study period, 89 patients with a mean age of 40 years underwent PGT-A. A total of 371 embryos were analyzed from 113 cycles, with 66% (245 embryos) resulting in aneuploidies. Approximately 94% of patients over 40 years old obtained at least one aneuploid embryo. The tables below show the classification of embryos in each study group, along with the percentage of different classifications, indicated within parentheses, in relation to the "Euploid" and "Aneuploid" groups.

	1 Nucleus	Binucleation	Multinucleation	Total
Euploid	97 (0.77)	11 (0.09)	18 (0.14)	**126 (1.0)**
Aneuploid	110 (0.45)	64 (0.26)	71 (0.29)	**245 (1.0)**

	Grade I	Grade II	Grade III	Grade IV	Total
Euploid	47 (0.37)	52 (0.41)	22 (0.18)	5 (0.04)	**126 (1.0)**
Aneuploid	4 (0.02)	78 (0.32)	140 (0.57)	23 (0.09)	**245 (1.0)**

The Chi-square test yielded a p-value of <0.001 for both nucleation and degree of fragmentation. These results indicate a significant association between the number of aneuploid embryos and nucleation variables, as well as fragmentation grades II and III. Similarly, a correlation was observed between euploid embryos and nuclear normality and fragmentation grade I. Regarding fragmentation grade, the analysis demonstrated a positive correlation with aneuploid embryos, indicating a direct relationship, while a negative correlation was observed with euploid embryos, indicating that higher fragmentation grades correspond to a lower number of euploid embryos. After the PGT-A results were released, 28% of patients did not undergo embryo transfer due to aneuploidy in all analyzed embryos, 16% had not undergone transfer at the time, 28% underwent transfer resulting in a positive Beta

hCG, 21% resulted in a negative Beta hCG, 2% experienced miscarriage, 5% had a biochemical pregnancy, and 0% had an ectopic pregnancy.

Conclusion: The results presented provide statistical evidence to reject the hypothesis of independence between nucleation and fragmentation grade variables in relation to PGT-A outcomes. Thus, increased fragmentation grade and nucleation abnormalities (binucleation or multinucleation) are strongly associated with an increased number of aneuploid embryos, corroborating to the literature. In the same way, nuclear normality and fragmentation grade I are strongly associated with an increased number of euploid embryos.