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Chromosomal status assessed by CGH after a day-3 biopsy, in embryos classified by standard scoring methodology at pronuclear, cleavage and blastocyst stage

Abstract

Background: The standard scoring methodology, combined with blastocyst culture remains a valuable tool for embryo selection. Nevertheless, the embryo assessment based exclusively on morphological traits does not provide information about the chromosomal status of the embryo.

Objective: The purpose of the present study has been to investigate the association between embryo morphology and chromosomal ploidy.

Materials and methods: A total of 196 embryos resulting of 30 PGS cycles were biopsied on day 3 and tested for aneuploidies by comparative hybridization array. The embryos were classified into groups according to their morphology at zygote, cleavage and blastocyst stage.

Results: Significantly statistical differences in embryo ploidy were observed when optimal day 3 morphology embryos were compared to medium morphology embryos (30.63% versus 17.28% respectively). A significantly higher proportion of euploid embryos were found in the group that achieved the blastocyst stage than in the arrested embryos group (42.57% versus 7.36%).

Conclusion: The results provide evidence that embryo morphology and aneuploidy are linked both at day 3 and blastocyst stage. However the chromosomal status of the embryo may not be predicted relying exclusively on morphological assessment.

Keywords: Standard embryo scoring; Aneuploidy; Preimplantation genetic screening; Comparative genomic hybridization

Volume 5 Issue 3 - 2017

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Received: July 24, 2017 | **Published:** September 7, 2017

Introduction

The standard scoring methodology, as the embryo progresses to the blastocyst stage, remains a valuable tool for embryo selection. Morphological parameters such as the percentage of fragments and cell size and number at cleavage stage are routinely examined, as morphologically optimal embryos have higher chances of resulting in pregnancy.[1]

Nowadays, couples with repeated implantation failures or miscarriages after day 3 transfers may benefit from prolonging embryo culture to blastocyst stage.[2,3] Numerous studies suggest a correlation between successful implantation and blastocyst quality indicated by the morphology of the inner cell mass and trophectoderm cells and the degree of expansion.[4,5] Additionally, blastocyst transfer permits better selection due to prolonged examination and exclusion of embryos that arrest at previous developmental stages.

Nevertheless, the selection of optimal embryos based on morphological criteria does not ensure the exclusion of chromosomally abnormal embryos.[6] The use of comparative genomic hybridization (CGH) array methodology in Preimplantation Genetic Screening (PGS) cycles enables a more thorough diagnosis of embryo chromosomal status and therefore the selection of euploid embryos for transfer.

Furthermore, by combining CGH findings with traditional embryo scoring it is possible to increase the knowledge on the impact of aneuploidy on each morphological trait at different developmental stages and to allow a more effective interpretation of embryo scoring in the future.The purpose of the study has been to investigate the association between embryo scoring and the chromosomal ploidy, as the latter is assessed after a day-3 biopsy by comparative genomic hybridization array.

Materials and methods

This is a retrospective observational study of 30 PGS cycles applied in 26 couples undergoing Controlled Ovarian Hyper stimulation/ICSI/Embryo-Transfer between the years 2012-2016 in EMBRYO A.R.T. Unit. A total of 196 biopsied embryos were included in the study. All participants had a PGS indication including advanced maternal age, recurrent implantation failures, recurrent abortions, and parental chromosomal translocations.

The patients underwent controlled ovarian hyper stimulation. Cycle monitoring was performed by transvaginal ultrasound examinations

and serum estradiol tests. Depending on the response of each patient, the dose of FSH was adjusted. Approximately 36hours after the administration of 10000 IU human chorionic gonadotrophin, the oocyte retrieval was performed through transvaginal ultrasonography.

After retrieval, the oocytes were incubated at 37°C in 6% CO_2 and 5% O2 for 2-3hours before locate denudation. All metaphase II oocytes were subjected to intracytoplasmic sperm injection (ICSI) under an inverted microscope (Olympus IX71) and returned to culture. On the following day, fertilization was confirmed by the presence of two pronuclei and two polar bodies. Normally fertilized oocytes were cultured in 50µl microdroplets of cleavage medium (Cook) overlaid with mineral oil for 48hours.

Only biopsies that were performed on day 3 were included in the study. On the third day of development, each embryo was placed in a 10µl biopsy medium droplet (Life Global) overlaid with mineral oil. A diode laser (Octax Laser System) was used to create a small opening in the zona pellucida 10-20µm wide. Only one blastomere with a single visible nucleus was removed from the embryo by using a biopsy tool. The embryo was returned to culture in blastocyst medium for 48hours. The blastomere was then transferred to a PCR tube and stored at -4°C. Testing was performed by array-comparative genome hydridization.

Embryos were classified into groups according to: zygote scoring (pronuclear stage), the number of cells/morphology/percentage of fragments (cleavage stage), and expansion status, quality of inner cell mass and trophectoderm cells (blastocyst stage). The quality of the ICM was graded as A (many cells, tightly packed), B (less cells, loosely grouped) or C (very few cells). According to the quality of the TE, the blastocysts were classified into the following groups: A (many cells forming a cohesive epithelium, B (few cells forming a loose epithelium) and C (very few large cells).

All embryos were biopsied at cleavage stage and chromosomal analysis was performed by array CGH. Chi-square test was used for statistical analysis. Fisher's exact test was performed to verify the results when statistically significant differences were observed.

Results

Mean (±SD) maternal and oocyte age was 36.83(±3.96) and 36.75(±4.58), respectively. Of the 196 biopsied embryos, 146 were diagnosed as aneuploid (74.48%) and 50 as euploid (25.52%). No statistically significant differences in embryo ploidy were observed when embryos were grouped according to pronuclear stage scoring; 18.18% of optimal quality zygotes were diagnosed as euploid versus 26.79% of medium quality ones (p=0.304).

Statistically significant differences in embryo ploidy were observed when embryos were grouped according to cleavage stage scoring; 30.63% of optimal quality embryos were diagnosed as euploid versus 17.28% of medium quality ones (p=0.028). Moreover, 101 out of 196 embryos (51.53%) that were biopsied on cleavage stage developed to blastocysts. A significantly higher proportion of euploid embryos was observed in the progressing embryos group (42.57%) compared to that in the arrested embryos group (7.36%), (p<0.001). 48.27% of blastocysts classified as class A in terms of the inner cell mass quality were euploid compared to 36.36% of those classified as of lower quality; the difference was not statistically significant (p= 0.06). No statistically significant differences regarding euploidy were observed among blastocysts classified as good, medium and poor according to the quality of trophectoderm cells (45.45%, 44.84% and 29.41%, respectively, p=0.102) (Tables 1&2).

Table 1 Comparison of euploidy rates according to day 1, day 3 and day 5 assessment

		Normal	Abnormal
	No of embryos	50(25.52%)	146(74.48%)
Day 1 Grading			
	Optimal	6(18.18%)	27(81.81%)
	Medium	41(26,79%)	113(73,21%)
	Poor	3(33.33%)	6(66.67%)
Chi-Square Test	P value	0.304	
Day 3 Grading			
	Optimal	34(30.63%)	77(69.36%)
	Medium	14(17,28%)	67(82,71%)
	Poor	2(33.33%)	4(66.67%)
Chi-Square Test	P value	0.028	
Fisher's Exact Test	P value	0.043	
Day 5 Grading			
	Blastocysts	43(42.57%)	58(57.42%)
	Arrested Embryos	7(7.36%)	88(92.63%)
Chi- Square Test	P value	<0.001	
Fisher's Exact Test	P value	<0.001	

Table 2 Comparison of euploidy rates according to the quality of the inner cell mass and the trophectoderm cells

	Normal	Abnormal
No of Blastocysts	43(42.57%)	58(57.42%)
Inner cell mass quality		
A	28(48.27%)	30(51.72%)
B	12(36.36%)	21(63.63%)
C	3(30.00%)	7(70.00%)
P value	0.06	
Trophectoderm cells quality		
A	25(45.45%)	30(54.54%)
B	13(44.84%)	16(55.16%)
C	5(29.41%)	12(70.59%)
P value	0.102	

Discussion

Earlier studies have suggested a potential link between morphology and aneuploidy.[7-9] The quality of the blastocyst has been associated with the results of comprehensive chromosome screening of trophectoderm biopsies.[10]

In the present study, embryos of optimal grade both at cleavage and blastocyst stage demonstrate a significantly higher probability of being diagnosed as euploid by CGH after day-3 biopsy. The

Citation: Anagnostopoulou C, Fligou M, Dinopoulou V, et al. Chromosomal status assessed by CGH after a day-3 biopsy, in embryos classified by standard scoring methodology at pronuclear, cleavage and blastocyst stage. *Endocrinol Metab Int J.* 2017;5(3):245–247. DOI: 10.15406/emij.2017.05.00126

results may provide evidence of a potential association between standard embryo scoring and euploidy, indicating the value of scoring methodology as an embryo selection tool for embryo-transfer when PGS is not applicable.

However, the retrospective design, the small sample size, and the relative subjectivity of embryo scoring remain the main limitations of the study. Additionally, the analysis based on a single blastomere biopsy at cleavage stage carries a higher risk of misdiagnosis due to mosaicism.

Conclusion

As a conclusion, further studies need to be conducted to confirm whether by thoroughly examining specific morphological traits of the preimplantation embryo it is possible to predict the chromosomal status of cleavage stage embryos and blastocysts and to minimize the risk of transferring aneuploid embryos.

Acknowledgments

None.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Funding

None.

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