NONINVASIVE PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDIES (niPGT-A): AGE X BLASTOCYST CHROMOSOMAL PLOIDY

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Introduction: Our objective was to study the characteristics of mosaicism chromosome of the human blastocyst, assessed by niPGT-A, in relation to increase age, as well as their degree of aneuploidy/euploidy detected by NGS genetic testing platform.

Material and Methods: This is a multicenter prospective study performed by ten assisted reproduction centers after training and validation process of the embryologists to use niPGT-A. A total of 94 couples with indication for niPGT-A due to increase maternal age, male factor, repeated implantation failures, recurrent abortion or because they requested niPGT-A were included in this work. All couples had no karyotype abnormalities. After ICSI, embryos were cultured until blastocyst stage using one or two step culture systems, single or sequential media respectively, at 37°C in an atmosphere of 6-7% CO2 and 5-20% O2 incubators. On day 3, cleavage embryos were re-evaluated to complete removal of the cumulus cells. Embryos were then cultured in an individual well with 20 µl of medium in GPS dishware under oil and cultured until they reach blastocyst stage. The blastocysts were vitrified and stored in liquid nitrogen. After that, the spent blastocyst culture medium (20µl) was transferred to a PCR tube and sent to the genetic analysis laboratory, where it was stored at -80°C until sequencing. A total of 220 samples of spent blastocyst culture medium were collected on the 5th/6th day. Cell-free DNA secreted on culture medium was amplified using NICS Sample Preparation Kit (Yikon Genomics), based on MALBAC technology. After whole genome amplification, the DNA was measured using a Qubit 2.0 fluorometer and subjected to next generation sequencing (NGS) using Illumina MiSeq® platform. The results were analyzed by ChromGo® software (Yikon Genomics).

Results: The mean age of the patients was 38±4.08 with an interval of 20-44 years. The euploid was diagnosed in 36.4% (80/220) of cases, aneuploidy in 31.3% (69/220), and mosaicism in 32.3% (71/220; with ≥60% aneuploidy) of blastocysts. Mosaic values ranged from 29.8% to 33.8% in different age groups (Table 1). Individually, the most frequent chromosomal abnormality was XXY (Klinefelter Syndrome) occurring in 18 cases, followed by chromosome 21 (trisomy/monosomy) in 8 cases (Table 2). niPGT-A data showed an ≥60% incidence of aneuploid cells in all cases of chromosomal mosaicism (n=71).

Table 1. Distribution of ploidy according to age rate

	Spent culture media (n=220)	Euploidy (n=80)	Aneuploidy (n=69)	Mosaicism* Aneuploidy/Euploidy (n=71)
<35 years	47	24 (51.1%)	9 (19.1%)	14 (29.8%)
35-37 years	32	14 (43.8%)	8 (25.0%)	10 (31.2%)
38-40 years	77	26 (33.8%)	25 (32.4%)	26 (33.8%)
41-42 years	45	12 (26.7%)	18 (40.0%)	15 (33.3%)
>42 years	19	4 (21.0%)	9 (47.4%)	6 (31.6%)

*Mosaics with ≥60% aneuploid cells

Table 2. Percentage of mosaicism and chromosomal abnormalities in different age groups

	<35 years	35-37 years	38-40 years	41-42 years	>42 years
Mosaicism* (n=71)	14	10	26	15	6
Chr 18 n=6	2 (14.3%)	1 (10%)	1 (3.8%)	1 (6.7%)	1 (16.7%)
Chr 21 n=8	1 (7.1%)	0	4 (15.4%)	2 (13.3%)	1 (16.7%)
Chr X0 n=2	0	0	2 (7.7%)	0	0
Chr XXY n=18	4 (28.6%)	2 (20%)	11 (42.3%)	1 (6.7%)	0
Chr Y0 n=4	2 (14.3%)	0	0	2 (13.3%)	0
Chr 14 n=2	0	1 (10%)	1 (3.8%)	0	0
Chr 15 n=5	1 (7.1%)	1(10%)	2 (7.7%)	1 (6.7%)	0
Chr 16 n=3	1(7.1%)	0	0	1 (6.7%)	1 (16.7%)
Chr 22 n=3	0	1 (10%)	0	1 (6.7%)	1 (16.7%)
Chr Others n=20	3 (21.5%)	4 (40%)	5 (19.3%)	6 (40%)	2 (33.3%)

*Mosaics with ≥60% aneuploid cells
Note: We did not have mosaics for chromosome

Conclusions: A high degree of mosaicism with aneuploidy cells was detected and some hypotheses were suggested for this data (sensitivity of niPGT-A in detecting the phenomenon of self-correction of chromosomal abnormalities). However, it did not vary remarkably with age. On the other hand, euploidy levels had a negative correlation with age and aneuploidy levels had a positive relationship. This is the first report in the literature to relate chromosomal ploidy in blastocysts using niPGT-A and increasing patient age.