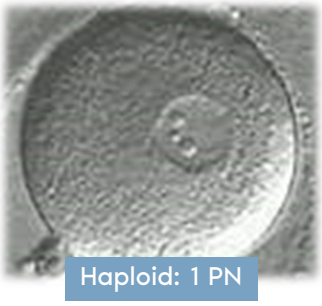
Summary of abstracts regarding ploidy assessment in blastocysts derived from normally and abnormally fertilized oocytes

Ploidy Check in Fertilized Oocytes

Diploid: 2 PN

Normally fertilized oocyte



Haploid: 1 PN

Triploid: 3 PN

Abnormally Fertilized Oocytes in IVF ~ 10%
These embryos are usually discarded due to their increased risk of ploidy abnormalities

ESHRE 39th Annual Meeting 2023

The incidence of different ploidy alterations in abnormally fertilized oocytes (AFO)-derived embryos

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Study question: Which is the incidence of de novo ploidy alterations in different abnormally fertilized embryos categories analysed with SNP-array? Summary answer: Based on the number of pronuclei (PN) identified, 0PN embryos were mostly diploid, while 1PN and >2PN-derived embryos showed increased rates of ploidy abnormalities.

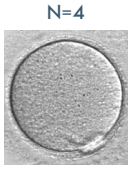
What is known already: Recent development of SNP-array and haplotyping by sequencing approaches are contributing to expanding preimplantation genetic testing (PGT) clinical utility including ploidy level evaluation. When applied to euploid embryos derived from AFO, the identification of abnormal ploidy constitution (i.e., haploidy, triploidy, or tetraploidy) could rescue viable diploid embryos otherwise not considered in IVF. However, no definitive genetic evidence has been obtained showing the incidence of different ploidy abnormalities in each AFO-derived blastocyst category. Here we present 4 years of experience in the clinical application of a validated SNP-array based protocol and custom-made algorithm to detect ploidy defects in AFO-derived embryos.

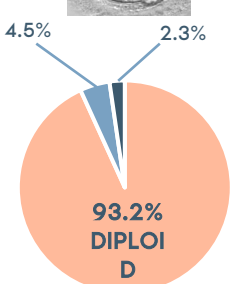
Study design, size, duration: Prospective observational study evaluating the incidence of altered ploidy configurations in AFO-derived blastocyst. After PN check, AFO samples were divided as follow: absence of observed pronuclei (0PN), monopronuclear (1PN), more than two pronuclei observed (2.1PN, with one smaller additional PN and 3PN). Genetic classification combining PGT-A and ploidy analysis was performed at Igenomix Italy laboratory between May 2019 and January 2023 on 133 AFO-derived embryos (44 0PN, 59 1PN, 30 >2PN).

Participants/materials, setting, methods: Multi-centre study involving 293 consenting patients of advance maternal age (mean=38.6 § 3.9) undergoing PGT-A on MDA-WGA using Ion ReproSeq kit and IonTorrent S5 (ThermoFisher). SNP-array-based ploidy test was performed using HumanKaryomap-12 kit and NextSeq550 (Illumina). Proprietary algorithm was based on genome-wide BAF obtained: expected BAFs were 1, 0.5 or 0 for diploids,1 or 0 for haploid and 1, 0.66, 0.33 or 0 for triploids, as determined by the frequency of each allele for 300000 SNPs loci.

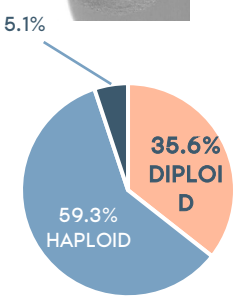
Results for ploidy analysis in abnormal fertilization observation

0PN-derived blastocysts
N=4

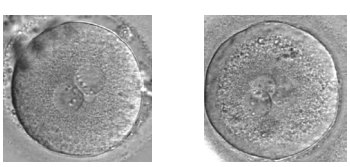


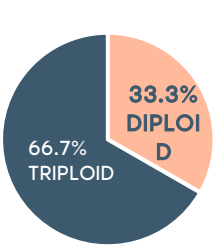


1PN-derived blastocysts
N=59



More than 2PN (2.1PN or 3PN) N=30





Ploidy test allowed the clinical use of 72 (out of 318) additional euploid/diploid embryos otherwise not considered for transfer

DIPLOID
HAPLOID
TRIPLOID

(Girardi et al., ESHRE 2023)

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Main results and the role of chance: Preclinical validation of SNP-arraybased ploidy test included 26 samples with known ploidy status and karyotype: 7 triploid re-biopsies, 7 haploid left-overs and 12 cell lines. Moreover, inter-platform comparison of 72 diploids, 9 triploids, 8 haploids embryos analysed with an alternative targeted-NGS approach, validated by Igenomix, showed 100% (95% CI= 95.94%-100%) concordance. In this study, a total of 318 AFO-derived embryos were collected for PGT-A analysis for different indications. Of these, 58.2% (n= 185/318; 95%CI=52.54-63.66) resulted as aneuploid. The remaining 133 euploid AFO-derived blastocyst where subjected to SNP-array based ploidy assessment. 0PN-derived blastocysts (n= 44/133) were diploid in 93.2% (n= 41/44; 95%CI=81.34-98.57) of cases; only 4.5% (n= 2/44; 95%CI=0.56- 15.47) were haploid and 2.3% (n= 1/44; 95%CI=0.06-12.02) were polyploid. 1PN-derived blastocysts showed all possible ploidy configurations in these proportions: 59.3% (n= 35/59; 95%CI=45.74-71.93) haploid, 35.6% (n= 21/59; 95%CI=23.55-49.13) diploid and 5.1% (n= 3/59; 95%CI=1.06-14.15) polyploid. Finally, in the group of AFO where more than 2PN were observed (2.1PN or 3PN), the additional PN resulted in 66.7% (n= 20/30; 95%CI=47.19-82.71) of polyploid configurations, the remaining 33.3% (n= 10/30; 95%CI=17.29-52.81) were diploid. No haploid results were obtained from this category. Chi-square test showed significant overall correlation between PN category and ploidy status configurations ($p < 0.05$). Collectively, ploidy evaluation of 133 AFO-derived embryos, allowed the clinical use of 72 additional euploid/diploid embryos otherwise not considered for transfer.

Limitations, reasons for caution: Ploidy assessment protocol was not tested to distinguish triploids from tetraploids. Parental origin of each chromosomal set could not be determined without analysing parental DNA. The way of performing PN check could have affected AFO classification. In future studies we will focus on evaluating clinical outcomes following euploid/diploid embryo transfer. Wider implications of the findings: An altered number of pronuclei is predictive of the correspondent altered ploidy status, while 0PN category doesn't represent a clear indication for ploidy evaluation. Nevertheless, a significant proportion of euploid/diploid embryos can be rescued from all types of AFO, potentially increasing the overall chance to achieve a live birth.

Trial registration number: n/a

ASRM Annual Meeting 2023

Determination of ploidy level integrated into the common preimplantation genetic testing for aneuploidy (PGT-A) and association with the pronuclei number

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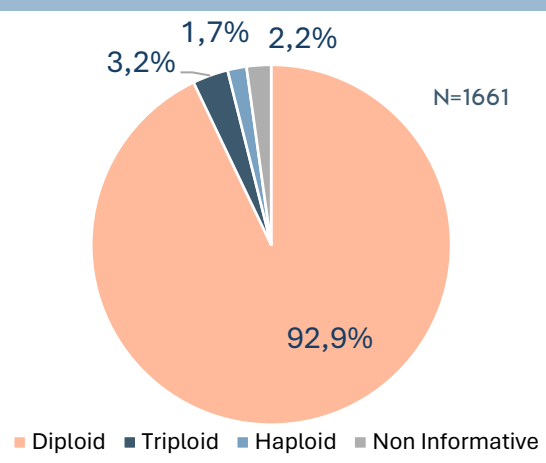
Objective: In vitro fertilization (IVF) is commonly combined with preimplantation genetic testing for aneuploidy (PGT-A) with the aim of detecting numerical and structural chromosomal abnormalities and minimizing the risks of transferring genetically abnormal embryos that may result in implantation failure and pregnancy loss, or congenital disorders in live-born offspring.

Although PGT-A is a reliable technique, it has limitations that do not allow the numerical alterations detection involving genome-wide ploidy. In order to determine the distribution of different levels of ploidy (haploidy, diploidy and triploidy) in trophectoderm biopsies screened in our laboratory, we used a customized ploidy test, based on a set of SNPs, as a complement to the PGT-A test.

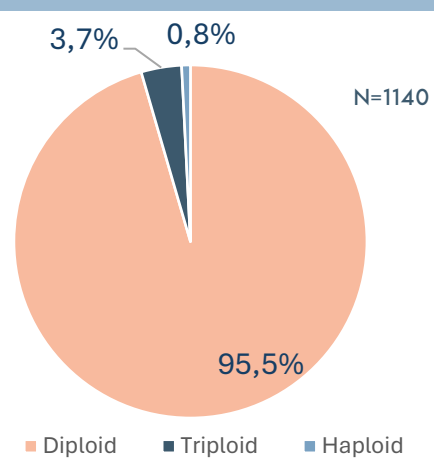
Materials and methods: A total of 7452 trohectoderm biopsies received between January and March 2023 in our laboratory for the PGTA test were evaluated. NGS-based PGT-A was performed on all biopsies by employing Whole Genome Amplification (WGA) protocol for shallow sequencing using Ion Reproseq PGS kit. Then, to infer the ploidy level of biopsies with euploid result in PGT-A, a panel of 357 biallelic SNPs with high allele frequencies located in regions consistently represented in the WGA protocol performed for PGT-A was used. A descriptive analysis of the distribution of ploidy levels in the biopsies was performed and the association of ploidy results with the morphological analysis of the number of pronuclei (PN) was verified by Fisher's exact test.

Results: Of the total number of biopsies analysed, 2868 showed euploid status in the PGT-A analysis and were processed to determine the ploidy level. The SNPs analysis showed 46 biopsies with a result consistent with haploidy (1.6%); 2640 diploid biopsies (92.05%); 105 triploid biopsies

Conventional Observation: 2PN Suspected



Time-lapse Observation: 2PN Confirmed



Ploidy test prevented the clinical use of 134 (4,7%) embryos with abnormal ploidy status that otherwise would be considered for transfer

(Copreski et al., ASRM 2023)

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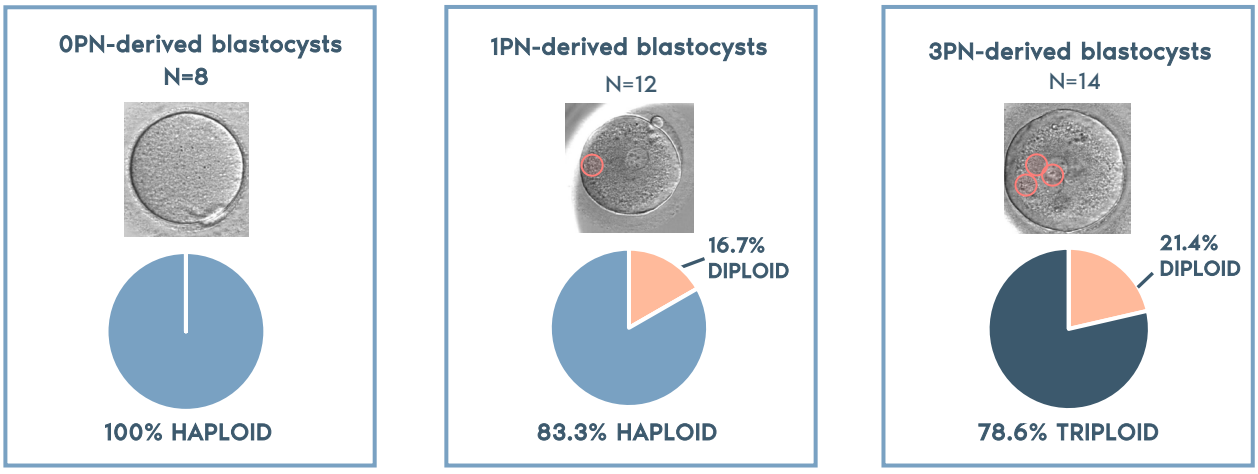
(3.66%) and 77 non-informative (2.69%). In close to half of the samples (1166), we had information of the pronuclei status and the biopsies were divided into the following groups i) Biopsies from two pronuclei (2PN)- derived blastocysts (n=1140), resulting in 1089 diploid (95.53%), 42 triploid (3.68%), 9 haploid (0.79%), ii) Biopsies from 0PN derived blastocysts (n=5), resulting all in a result consistent with haploidy, iii) Biopsies from 1PN derived blastocyst (n=10), resulting in 8 haploid (80%) and 2 diploid biopsies (20%), and iv) Biopsies from R3PN (n=11), resulting in 9 triploid (81.8%) and 2 diploid biopsies (18.2%). A significant association was detected between the variables ploidy status and the standard morphological assessment of PN.

Conclusions: Trophectoderm biopsies with euploid results in the common PGT-A test may show an altered ploidy level. Despite the association between ploidy status and PN, our results showed that the morphological analysis of the PN is not sufficient to rule out the chances of altered ploidy.

Impact Statement: The detection of aneuploidies accompanied by the determination of the ploidy status yields a more comprehensive view of the embryo genomic composition and improves the general clinical utility of preimplantation genetic testing for infertile couples.

Time-lapse Observation: 0PN, 1PN and 3PN Confirmed



Ploidy test allowed the clinical use of **5 (out of 26)** diploid embryos otherwise not considered for transfer

(Copreski et al., ASRM 2023)

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