Incidence of ploidy abnormalities according to PGT-A indication in embryos derived from normally fertilised oocytes

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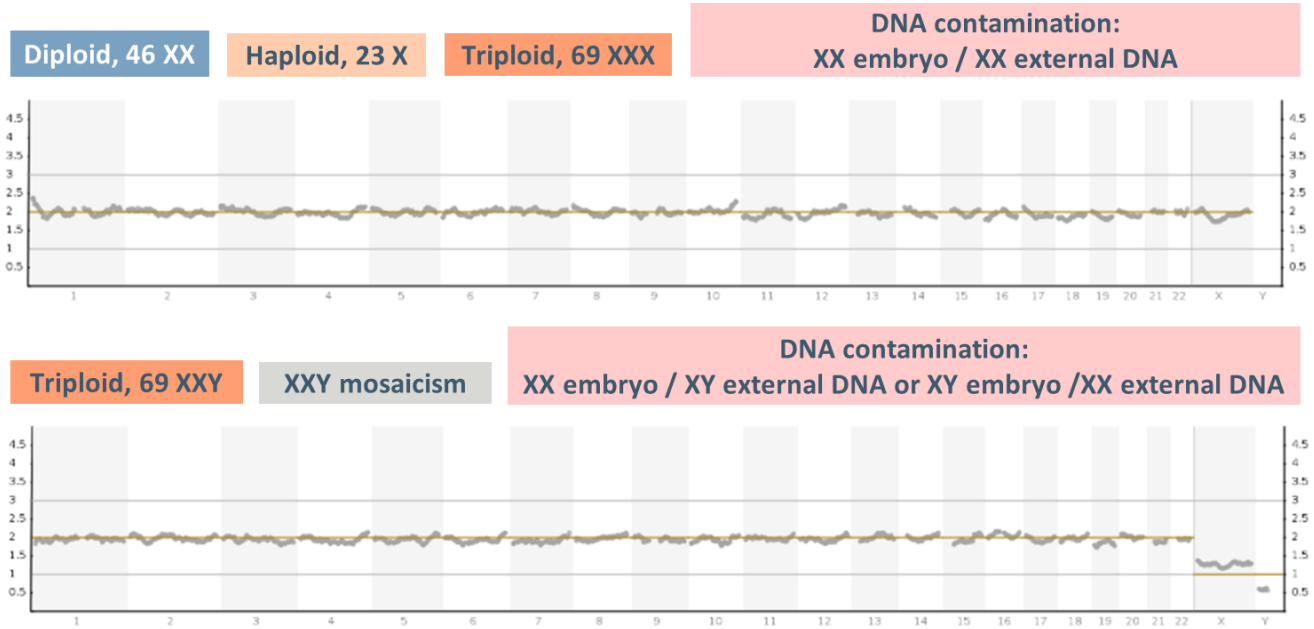
Ploidy assessment in PGT-A

PGT-A NGS-based platforms compare chromosome copy number (CN) ratios to identify aneuploides (chromosome gain/losses), but **cannot distinguish variations in the number of complete chromosome sets** (ploidy)



Euploid (XX)

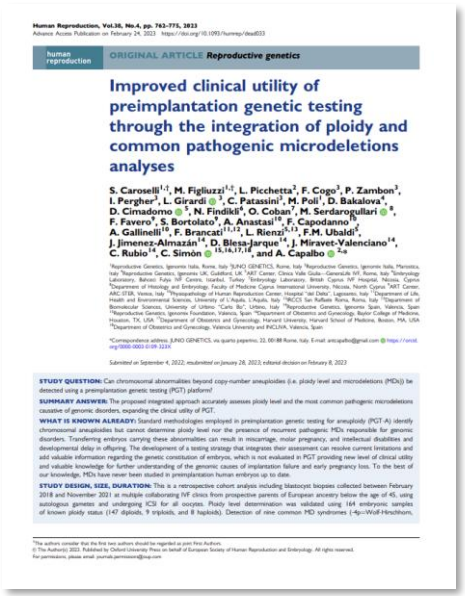
Intermediate copy number for sex chromosomes

Haploidy and triploidy are both compatible with preimplantation embryonic development.

Triploidy can be found in 10% of chromosomally abnormal spontaneous miscarriages and is usually not compatible with development to term *(Boué et al., 1975; Hassold et al., 1980; Plachot et al., 1989, Strom et al., 1990)*.

Ploidy assessment in PGT-A

Incorporating **SNP analysis to the NGS-based PGT-A workflow** allows the integration of **genotype information** and copy-number analysis to incorporate **ploidy assessment** within a standard PGT-A workflow


Human Reproduction, Vol.38, No.4, pp. 762–775, 2023

human reproduction ORIGINAL ARTICLE Reproductive genetics

Improved clinical utility of preimplantation genetic testing through the integration of ploidy and common pathogenic microdeletions analyses

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GENETICS

Incidence of haploidy and triploidy in trophectoderm biopsies of blastocysts derived from normally and abnormally fertilized oocytes

Laura Girardi[1] · Cristina Patassini[1] · Jose Miravet Valenciano[2] · Yoshimi Sato[2] · Natalia Fagundes Cagnin[4] · Jose Antonio Castellón[3] · Francesco Cogo[3] · Paola Zambon[3] · David Biesa[2] · Jorge Jimenez Almazan[2] · Adedoyin Akinwole[5] · Bruno Coprerski[4] · Carmen Rubio[4]

Received: 25 July 2024 / Accepted: 24 September 2024

Abstract

Purpose We aimed to identify the correlation between morphological pronuclear (PN) status and the genetically determined ploidy configuration in preimplantation embryos.

Methods A retrospective observational study was conducted on 1982 embryos displaying normal fertilization and 380 embryos showing an atypical PN pattern, tested for aneuploidies and ploidy status via preimplantation genetic testing (PGT) between May 2019 and May 2024. Ploidy prediction was performed using a validated targeted-NGS approach and a proprietary bioinformatic pipeline analyzing SNPs B-allele frequency information. Ploidy results were obtained in relation to the morphological PN pattern and further stratified by mode of PN observation, maternal age, and embryo quality parameters.

Results Abnormal ploidy results in 2PN-derived embryos were 1% (n = 20/1982); 0.8% showed triploidy and 0.2% haploidy. Ploidy results in relation to PN number in atypical fertilization were as follows: 0PN (n = 150/380) associated with 87.3% of diploidy, 8.7% of haploidy, and 4.0% of triploidy; 1PN-derived blastocysts (n = 73/153) were haploid in 47.7% of cases, 6.5% were triploid, and 45.7% diploid; 2.1PN (n = 23/280) and 3PN patterns (n = 54/280) predicted a triploid result in 34.8% and 74.1% of cases, respectively. PN observation with time-lapse increased ploidy status predictivity from 28.3% to 80.4% ($p < 0.01$) and reduced expected diploid rates to 19.6% ($p < 0.01$). Diploidy rate was higher for maternal age ≤ 35 years for morphologically high-grade embryos.

Conclusion Morphological PN check can be improved by incorporating ploidy analysis within the conventional PGT workflow. Euploid 2PN-derived embryos can be further selected removing haploids and triploids, and some atypical PN pattern can be better classified.

Keywords Ploidy · Pronuclei · PGT · NGS · Fertilization · Time-lapse

Introduction

Normal fertilization, defined as the presence of two pronuclei and two polar bodies, is generally assessed in the in vitro fertilization (IVF) setting by microscopic evaluation of inseminated oocytes between 16- and 18-h post-fertilization [1]. Oocytes without exactly two pronuclei (PN)—0PN (non-pronuclear), 1PN (mono-pronuclear), or more than 2PN configurations—are considered abnormally fertilized (https://atlas.eshre.eu/es/1454665024811190) and not recommended for clinical use [1]. The practice of excluding embryos with abnormal PN patterns is usually justified based on the increased risk of unbalanced genomic contribution from the gametes, uniquely predicted by PN

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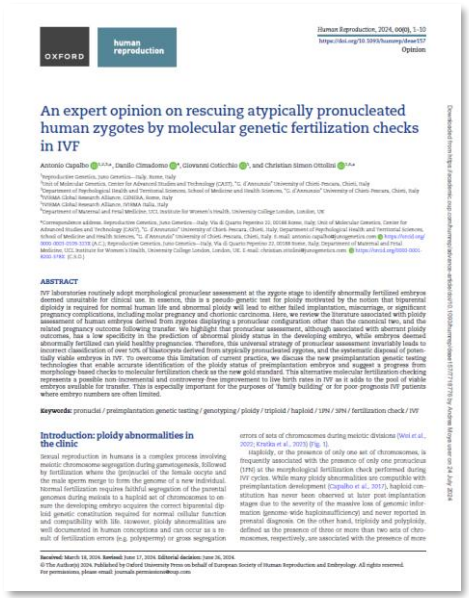
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https://doi.org/10.1093/humrep/dear157
Opinion

An expert opinion on rescuing atypically pronucleated human zygotes by molecular genetic fertilization checks in IVF

Antonio Capalbo[1,2,3,4,*], Danilo Cimadomo[5,6], Giovanni Coticchio[7,8], and Christian Simon Ottolini[1,2,4,*]

ABSTRACT

IVF laboratories routinely adopt morphological pronuclear assessment at the zygote stage to identify abnormally fertilized embryos deemed unsuitable for clinical use. In essence, this is a pseudo-genetic test for ploidy motivated by the notion that biparental diploidy is required for normal human life and abnormal ploidy will lead to either failed implantation, miscarriage, or significant pregnancy complications, including molar pregnancy and chorionic carcinoma. Here, we review the literature associated with ploidy assessment of human embryos derived from zygotes displaying a pronuclear configuration other than the canonical two, and the related pregnancy outcome following transfer. We highlight that pronuclear assessment, although associated with aberrant ploidy outcomes, has a low specificity in the prediction of abnormal ploidy status in the developing embryo, while embryos deemed abnormally fertilized can yield healthy pregnancies. Therefore, this universal strategy of pronuclear assessment invariably leads to incorrect classification of over 50% of blastocysts derived from atypically pronucleated zygotes, and the systematic disposal of potentially viable embryos in IVF. To overcome this limitation of current practice, we discuss the new preimplantation genetic testing technologies that enable accurate identification of the ploidy status of preimplantation embryos and suggest a progress from morphology-based checks to molecular fertilization check as the new gold standard. This alternative molecular fertilization-checking represents a possible non-incremental and controversy-free improvement to live birth rates in IVF as it adds to the pool of viable embryos available for transfer. This is especially important for the purposes of 'family building' or for poor-prognosis IVF patients where embryo numbers are often limited.

Keywords: pronuclei / preimplantation genetic testing / genotyping / ploidy / triploid / haploid / 1PN / 3PN / fertilization check / IVF

Introduction: ploidy abnormalities in the clinic

Sexual reproduction in humans is a complex process involving meiotic chromosome segregation during gametogenesis, followed by fertilization when the haploidy of the female oocyte and the male sperm merge to form the genome of a new individual. Normal fertilization requires faithful segregation of the parental genomes during meiosis to a haploid set of chromosomes to ensure the developing embryo acquires the correct biparental diploid genetic constitution required for normal cellular function and compatibility with life. However, ploidy abnormalities are well documented in human conceptions and can occur as a result of fertilization errors (e.g. polyspermy) or gross segregation errors of sets of chromosomes during meiotic divisions (Wei et al., 2022; Kristiko et al., 2023) (Fig. 1).

Haploidy, or the presence of only one set of chromosomes, is frequently associated with the presence of only one pronucleus (1PN) at the morphological fertilization check performed during IVF cycles. While many ploidy abnormalities are compatible with preimplantation development (Capalbo et al., 2017), haploid constitution has never been observed at later post-implantation stages due to the severity of the massive loss of genomic information (genome-wide haploinsufficiency) and never reported in prenatal diagnosis. On the other hand, triploidy and polyploidy, defined as the presence of three or more than two sets of chromosomes, respectively, are associated with the presence of more

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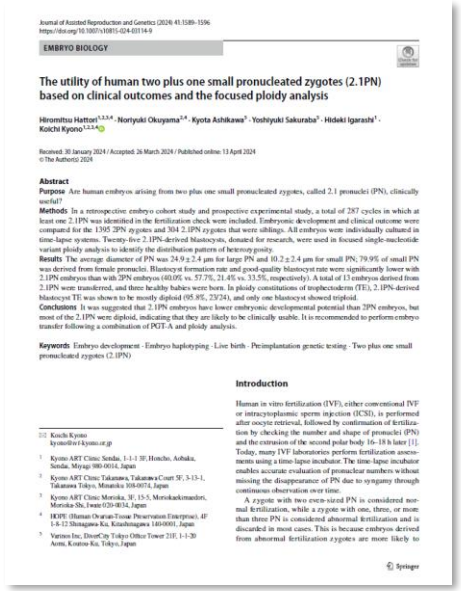
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EMBRYO BIOLOGY

The utility of human two plus one small pronucleated zygotes (2.1PN) based on clinical outcomes and the focused ploidy analysis

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Abstract

Purpose Are human embryos arising from two plus one small pronucleated zygotes, called 2.1 pronuclei (PN), clinically useful?

Methods In a retrospective embryo cohort study and prospective experimental study, a total of 287 cycles in which at least one 2.1PN was identified in the fertilization check were included. Embryonic development and clinical outcome were compared for the 1395 2PN zygotes and 304 2.1PN zygotes that were siblings. All embryos were individually cultured in time-lapse systems. Twenty-five 2.1PN-derived blastocysts, donated for research, were used in focused single-nucleotide variant ploidy analysis to identify the distribution pattern of heterozygosity.

Results The average diameter of PN was 24.9 ± 2.4 μm for large PN and 10.2 ± 2.4 μm for small PN; 79.9% of small PN was derived from female pronuclei. Blastocyst formation rate and good-quality blastocyst rate were significantly lower with 2.1PN embryos than with 2PN embryos (40.0% vs. 57.7%, 21.4% vs. 33.5%, respectively). A total of 13 embryos derived from 2.1PN were transferred, and three healthy babies were born. In ploidy constitutions of trophectoderm (TE), 2.1PN-derived blastocyst TE was shown to be mostly diploid (95.8%, 23/24), and only one blastocyst showed triploid.

Conclusions It was suggested that 2.1PN embryos have lower embryonic developmental potential than 2PN embryos, but most of the 2.1PN were diploid, indicating that they are likely to be clinically usable. It is recommended to perform embryo transfer following a combination of PGT-A and ploidy analysis.

Keywords Embryo development · Embryo haplotyping · Live birth · Preimplantation genetic testing · Two plus one small pronucleated zygotes (2.1PN)

Introduction

Human in vitro fertilization (IVF), either conventional IVF or intracytoplasmic sperm injection (ICSI), is performed after oocyte retrieval, followed by confirmation of fertilization by checking the number and shape of pronuclei (PN) and the extrusion of the second polar body 16–18 h later [1]. Today, many IVF laboratories perform fertilization assessments using a time-lapse incubator. The time-lapse incubator enables accurate evaluation of pronuclear numbers without missing the disappearance of PN due to syngamy through continuous observation over time.

A zygote with two even-sized PN is considered normal fertilization, while a zygote with one, three, or more than three PN is considered abnormal fertilization and is discarded in most cases. This is because embryos derived from abnormal fertilization zygotes are more likely to


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MDPI

Article

The Identification of Molecular Ploidy Status of Abnormal Pronuclear Zygotes Reveals a Significant Number of Euploid Blastocysts Available for Conception

Blair R. McCallie *, Mary E. Haywood, Lauren N. Henry, Rachel M. Lee, William B. Schoolcraft and Mandy G. Katz-Jaffe

Abstract: Objective: Abnormally fertilized embryos are often discarded during in vitro fertilization due to the fact that known chromosomal ploidy abnormalities lead to implantation failure or pregnancy loss. The objective of this study was to determine if pronuclear numeration (PN) observed at fertilization check is representative of the true ploidy status of the subsequent developing blastocyst in order to maximize the number of viable embryos available for infertility patients and increase their chances of conception. Methods: Upon successful fertilization, pronuclear numeration was noted, and zygotes were cultured to the blastocyst stage. Biopsied trophectoderm cells were then lysed, and the isolated DNA was whole-genome amplified followed by library preparation. Next-generation sequencing was performed for PGT-A, and excess whole-genome amplified DNA was utilized for single nucleotide polymorphism beadchip array analysis. Results: At the time of fertilization check on day 1 of embryo development, when there were no visible pronuclei (n = 291), 56% of these 0PN blastocysts were confirmed to be diploid and normally fertilized. The remaining 41.9% were aneuploid, and 2.1% of the 0PN blastocysts contained only 23 haploid chromosomes. Upon analysis of the 1PN blastocysts (n = 217), just over a third (36.4%) only contained 23 haploid chromosomes (23XO), with another third (31.8%) identified as aneuploid, and surprisingly, the remaining third (31.8%) confirmed to be diploid and normally fertilized. In contrast, 50% of the 3PN blastocysts (n = 172) showed the presence of a third set of 23 parental chromosomes and were confirmed to be triploid (69XXY = 59.3% and 69XXX = 40.7%), with 41.9% identified as aneuploid and, interestingly, a small percentage (8.1%) confirmed to be diploid with normal fertilization. A very small proportion of biopsied blastocysts (0.63%) displaying the correct number of pronuclei for normal fertilization (2PN) were also identified as triploid with a third set of 23 parental chromosomes. To date, there have been 74 euploid embryo transfers from zygotes originally identified with an alternate pronuclear numeration, resulting in 16 ongoing pregnancies and 32 healthy live births, rates that match those typically observed with normally fertilized 2PN zygotes (>60%). Conclusions: A surprising number of blastocysts that were identified to have alternate pronuclear numeration at fertilization check on day 1 of embryo development were actually determined to be diploid with normal fertilization after molecular analysis. Accurate identification of haploid and triploid zygotes is critical to prevent implantation failure and pregnancy loss and allows for the identification of all euploid embryos in a cohort, which has the potential to increase cumulative live birth rates for infertility patients.

Keywords: pronucleus; abnormal fertilization; infertility; pregnancy loss

Incidence of haploidy and triploidy in abnormally fertilized oocytes

Journal of Assisted Reproduction and Genetics
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GENETICS

Incidence of haploidy and triploidy in trophectoderm biopsies of blastocysts derived from normally and abnormally fertilized oocytes

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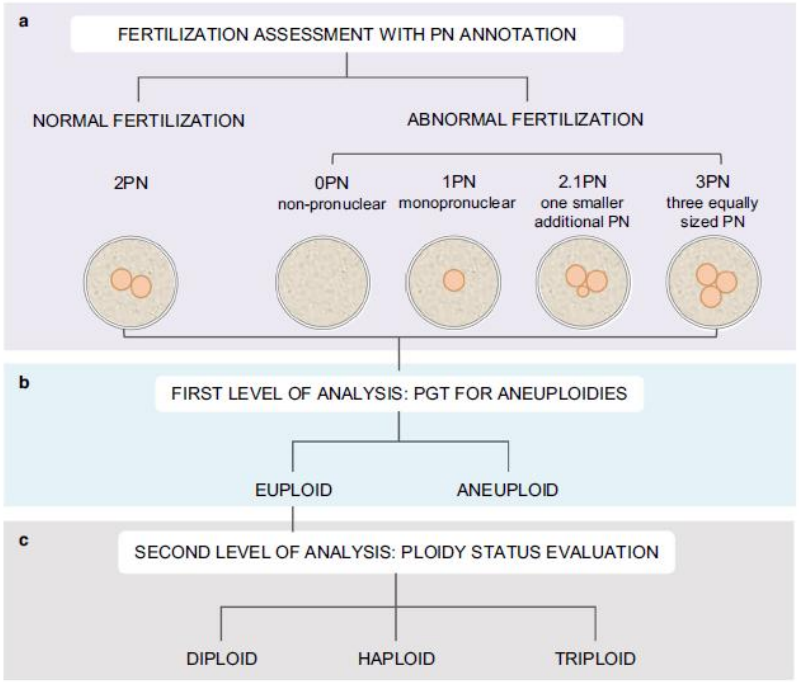

Conventional static PN evaluation

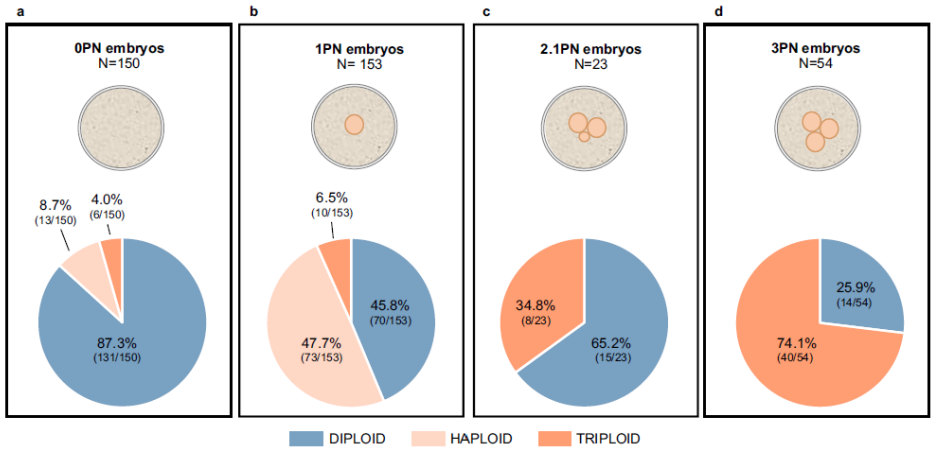

Fig. 4 Global distribution of ploidy configurations in abnormally fertilized zygotes with different pronuclear statuses. Overview of ploidy results distribution (diploid, haploid, and triploid) reported for each group of embryos concerning the number of pronuclei (PN) detected. Ploidy results in **a** 0PN-derived embryos, **b** 1PN-derived embryos, **c** 2.1PN-derived embryos, and **d** 3PN-derived embryos

PN evaluation by time-lapse monitoring system

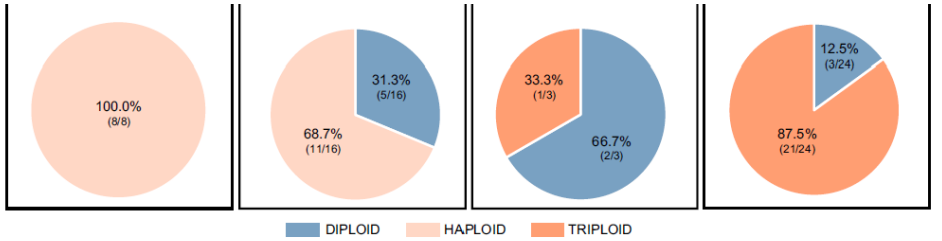

Fig. 5 Ploidy constitution of abnormally fertilized zygotes confirmed by time-lapse observation. Overview of ploidy result distribution (diploid, haploid, and triploid) reported for **a** 0PN, **b** 1PN, **c** 2.1PN, and **d** 3PN-derived embryos when identifying pronuclear (PN) status with time-lapse instruments

Although time-lapse monitoring systems improve the assessment of ploidy status in embryos with atypical PN patterns, SNP analysis identifies some of them as diploid and therefore, potentially transferable

Incidence of haploidy and triploidy in normally fertilized oocytes

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2024

GENETICS

Incidence of haploidy and triploidy in trophectoderm biopsies of blastocysts derived from normally and abnormally fertilized oocytes

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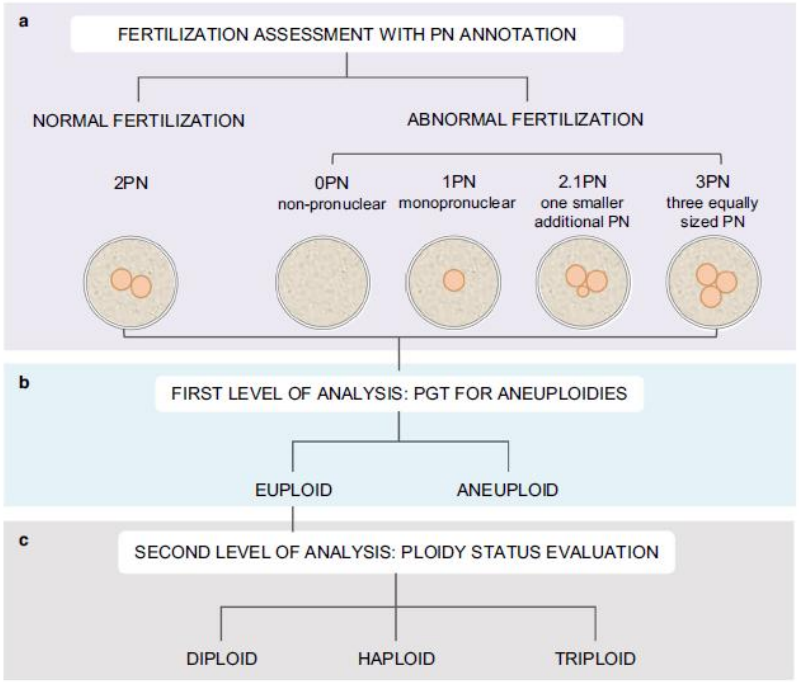


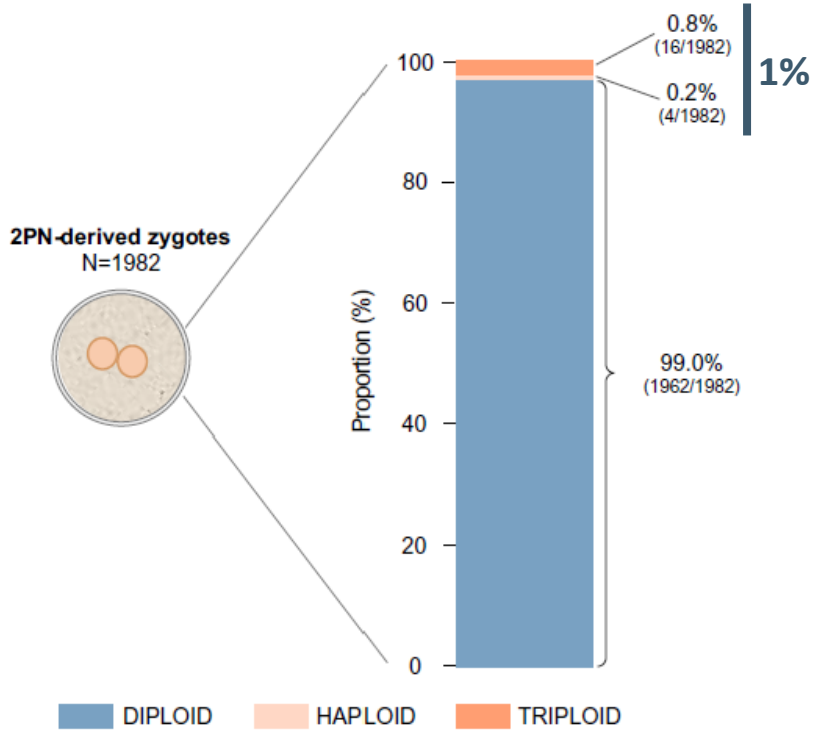


Fig. 2 Global distribution of ploidy results in normally fertilized zygotes. Ploidy status distribution in 1982 2PN-derived blastocysts or presumed normally fertilized embryos

SNP analysis identifies 1% of 2PN embryos with abnormal ploidy (triploid and haploid), allowing them to be deselected for transfer and potentially increasing reproductive outcomes

Igenomix®

Objectives

- ✓ To evaluate the incidence of abnormal ploidy in embryos derived from normally fertilised oocytes (2PN).
- ✓ To evaluate the impact of different PGT-A indications on ploidy results.
- ✓ To evaluate the impact of female age on ploidy results.

Material and methods

Retrospective study (November 2023 – November 2024) in couples performing **Smart PGT-A Plus**
(analysed at Igenomix USA, Canada, Brazil and Spain)
Ploidy, contamination and cohort testing in embryos derived from **normally fertilised oocytes (2PN)**

Samples

3,136 embryos

Female age ≤ 35

Good Prognosis (GP)	Male Factor (MF)	Recurrent Pregnancy Loss (RPL)	Repetitive Implantation Failure (RIF)
1,767	301	141	43

Female age > 35

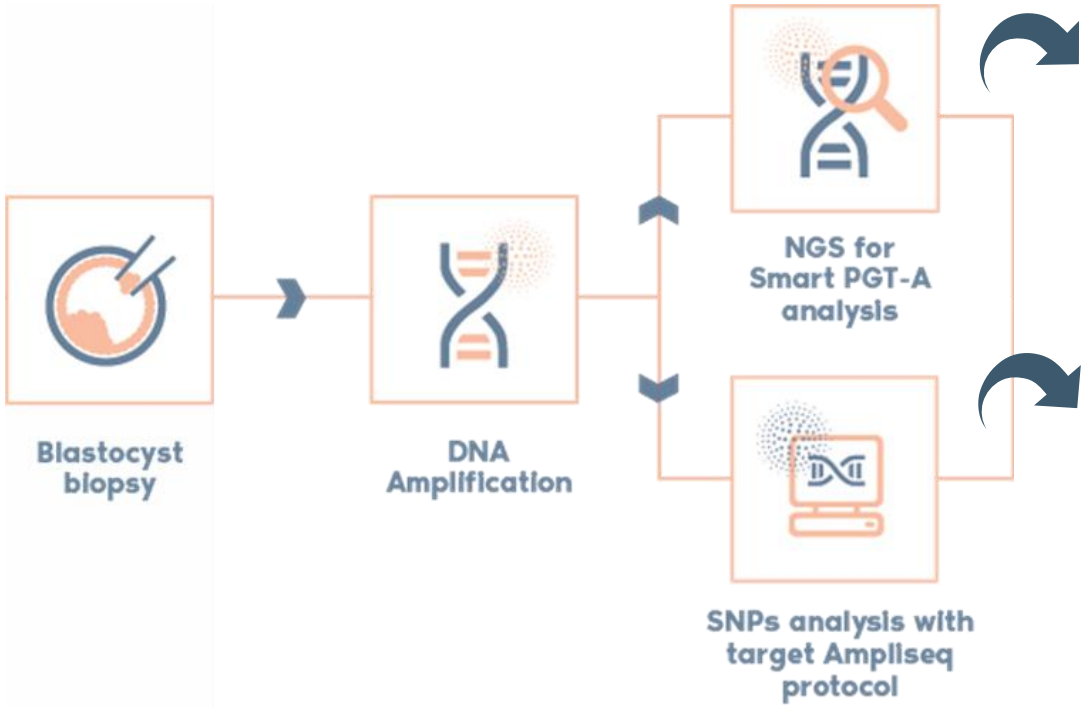
Advanced Maternal Age (AMA)	
884	

≤ 40 years	> 40 years
529	355

Material and methods

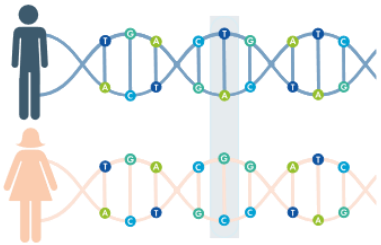
Smart PGT-A Plus

SNP analysis in PGT-A workflow



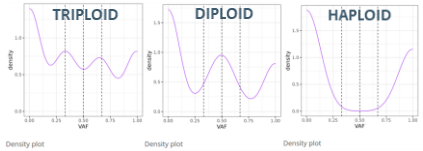
Ion Reproseq PGS kit and a proprietary bioinformatics pipeline (v2.0) were used for 24-chromosome aneuploidy testing.

Custom Ion AmpliSeq panel of **357 selected SNPs**
(Thermo Fisher Scientific, USA)



Proprietary algorithm for data analysis (v1.0)

Ploidy detection
(triploidy, haploidy)

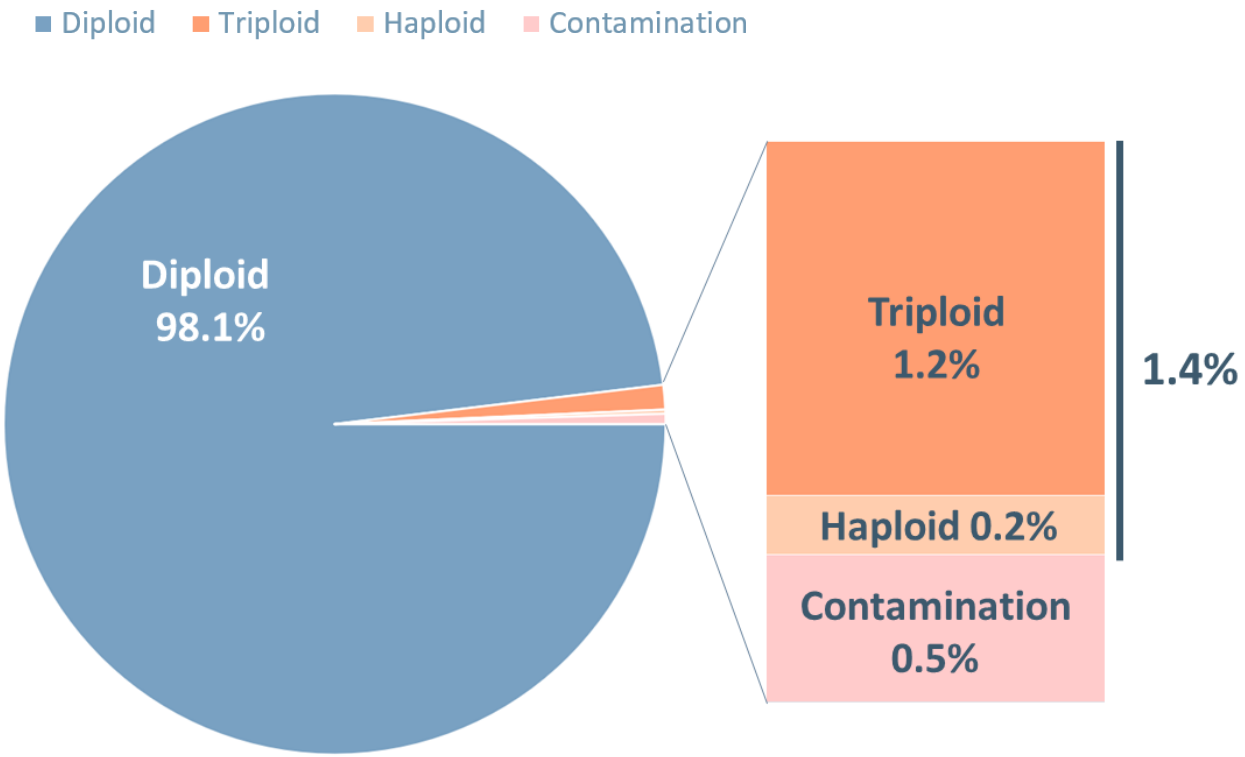


DNA contamination
(external cell/DNA and maternal cell contamination)

Cohort check
(genetic relatedness between embryos in a cohort)

Results

Incidence of abnormal ploidy in embryos derived from normally fertilised oocytes (2PN)



2,841 embryos informative for Ploidy and Contamination

1,825 embryos considered for Cohort testing
(cohort with 2 or more analysable embryos)

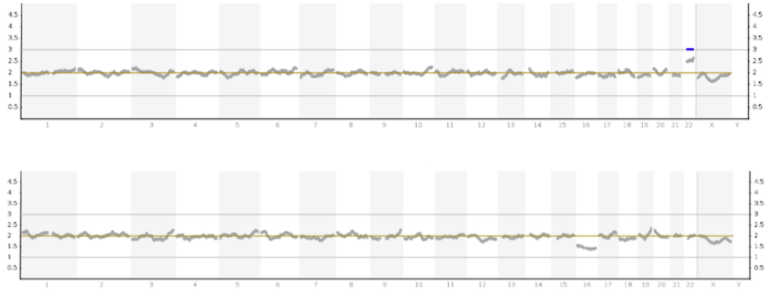
- Informative for cohort testing: 99.5%
- All the informative embryos showed genetic relatedness to their cohort

Results

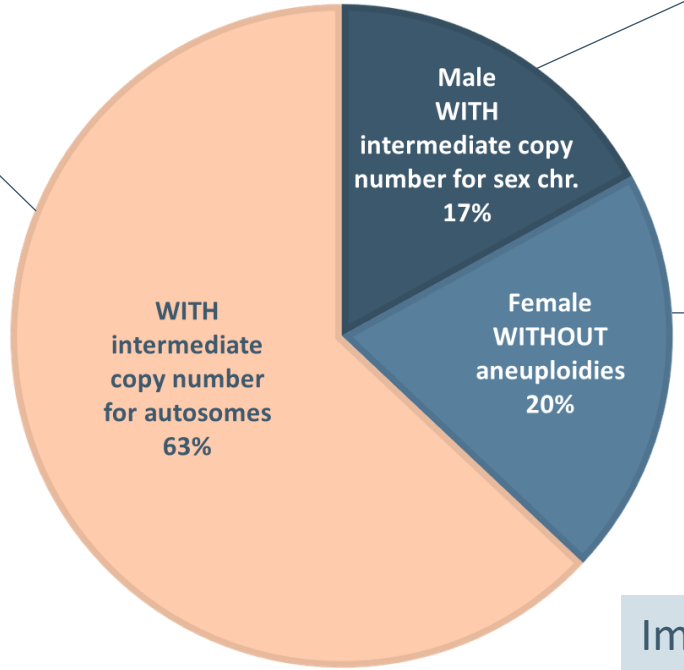
Incidence of abnormal ploidy in embryos derived from normally fertilised oocytes (2PN)

1.4% (41/2827) TRIPLOID / HAPLOID EMBRYOS

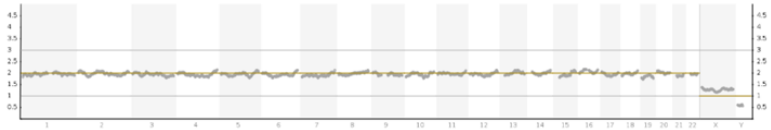
Mosaic
for PGT-A without SNP



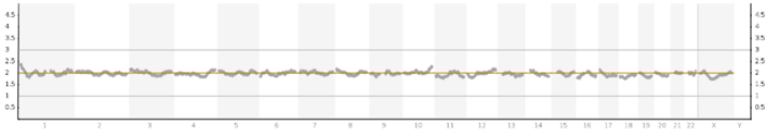
More accurate mosaicism diagnosis



Intermediate copy number for sex chr.
for PGT-A without SNP



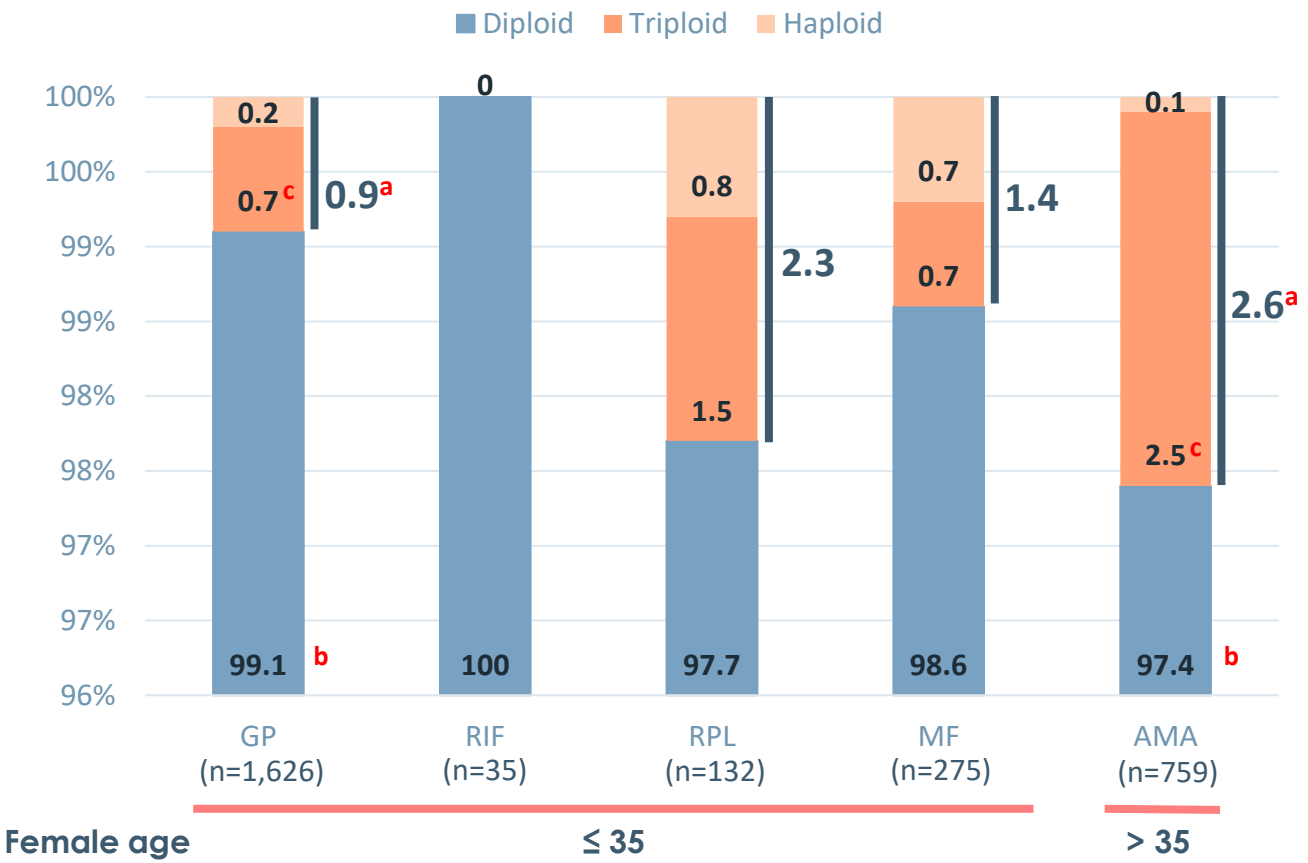
Euploid XX
for PGT-A without SNP



Improved accuracy of PGT-A by identification of triploidy/haploidy in embryos otherwise diagnosed as euploid XX

Results

Incidence of abnormal ploidy according to PGT-A indication



a-a p<0.01; b-bp<0.05; c-c p<0.001; Fisher's exact test vs GP

Embryos with abnormal ploidy:

- Significantly increased incidence in AMA vs GP
- Higher trend in RPL and MF vs GP
- Similar incidence in RIF vs GP

Triploid embryos:

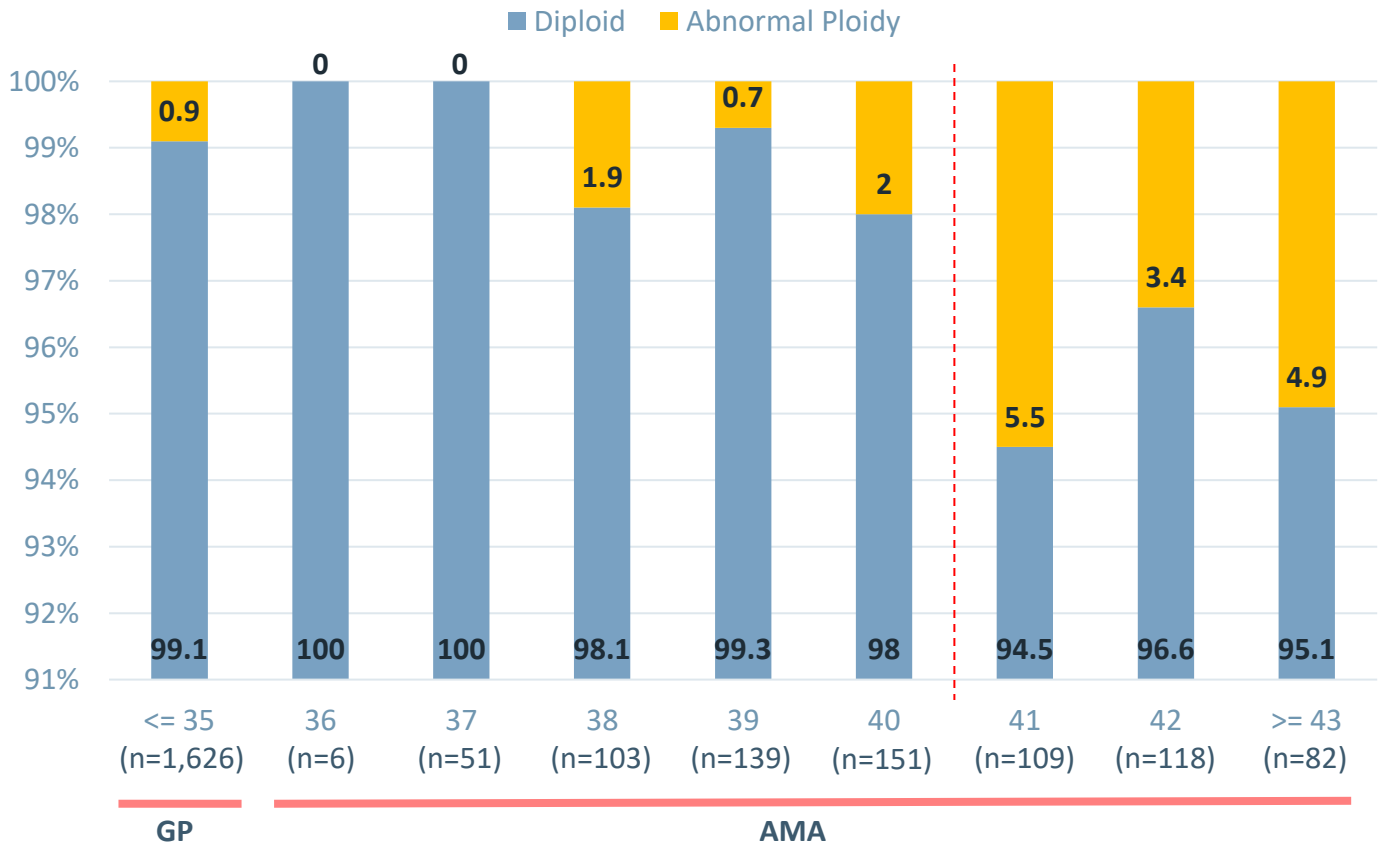
- Significantly increased incidence in AMA vs GP
- Higher trend in RPL vs GP
- Similar incidence in MF, RIF and GP

Haploid embryos:

- Higher trend in RPL and MF vs GP
- Similar incidence in AMA, RIF and GP

Results

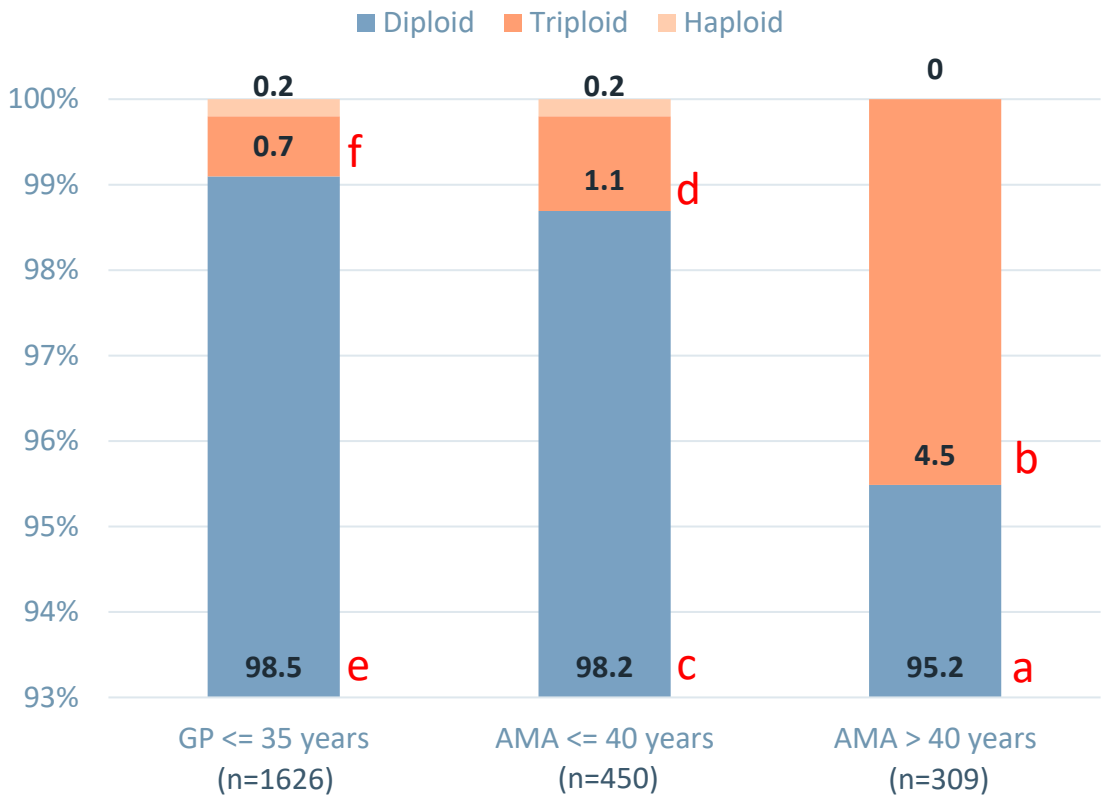
Incidence of abnormal ploidy according to female age



An increase in the number of embryos with abnormal ploidy is observed with the female age, which becomes more pronounced in women >40 years of age

Results

Incidence of abnormal ploidy in two age groups



a-c $p<0.05$; b-d $p<0.01$; a-e, b-f $p<0.001$; Fisher's exact test

Triploid embryos:

- Significantly higher incidence in AMA >40 vs. AMA ≤40 and GP
- Similar incidence in AMA ≤40 years and GP

Haploid embryos:

- Similar incidence in AMA >40, AMA ≤40 and GP

Conclusions

1. **1.4% of blastocyts** derived from normal fertilization (2PN) were identified as **triploid or haploid**.

2. Patients with AMA (>35 years) had the highest incidence of embryos with abnormal ploidy (2.6%), mainly related to triploidy, and this was more prevalent in woman >40 yrs (4.5%).

3. Young patients (≤35) with clinical history of RPL (2.3%) and MF (1.4%) showed particularly high rates of triploid or haploid embryos compared to good prognosis patients (0.9%).

4. PGT-A with **ploidy and contamination analysis decreases the risk of misdiagnosis** by identifying samples with non-embryonic DNA contamination and improving the diagnosis of mosaicism and embryos with intermediate sex chromosomes copy number changes.

The incorporation of SNP analysis in PGT-A results in a more accurate diagnosis with potential improvements of clinical outcomes. Our results indicate the higher impact in patients >40 yrs and in RPL patients.

Incidence of ploidy abnormalities according to PGT-A indication in embryos derived from normally fertilised oocytes

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