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Chapter

Aneuploidy Rates Inversely Correlate with Implantation during *In Vitro* Fertilization Procedures: In Favor of PGT

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Abstract

Aneuploidy, the hold of an abnormal number of chromosomes that differs from the normal karyotype, is a recognized leading cause of miscarriage and congenital disabilities. In human gametes and embryos, aneuploidy rates are prevalent, and these rates increase with advanced maternal age; additionally, it has been suggested that hormonal stimulation for achieving *in vitro* fertilization (IVF) protocols further increases aneuploidy rates. Although about 65% of chromosomally abnormal embryos culminate in spontaneous miscarriages, there is still evidence of live births harboring crucial aneuploidies. Furthermore, although some frequent aneuploidies are consistent, others differ between countries, making it harder to focus on a specific set of anomalies but vital to focus regionally on those more prevalent. Preimplantation genetic testing (PGT) is a highly endorsed technique in assisted reproductive treatments to evaluate possible embryo aneuploidies, genetic defects, and congenital disorders. On this subject, this study shows that IVF aneuploidy rates in embryo cohorts of high morphological quality are inversely associated with implantation rates. In its entirety, this study reinforces the utility of PGT for embryo evaluation.

Keywords: aneuploidy, preimplantation genetic testing, embryo implantation, *in vitro* fertilization, karyotype

1. Introduction

Aneuploidy is defined as a chromosome number that is not an exact multiple of the usually haploid number [1]. The terms haploid and diploid that describe single (n) and double (2n) chromosome sets in cells originate from the Greek terms *haplóos* meaning single and *diplóos* meaning double. The term ploidy was subsequently derived to describe the total chromosome content of cells. Consequently, the term euploid refers to a chromosome with an exact multiple of the haploid number [2]. Human body cells (somatic cells) are diploid, carrying two complete sets of chromosomes: one set of 23 chromosomes from their father and one set of 23 chromosomes from their mother; the two sets combined provide a full complement

of 46 chromosomes. Human gametes (or sex cells), sperm and oocytes, are haploid and contain only one set of 23 chromosomes.

Aneuploidies can occur either by chromosome gains (trisomies) and losses (monosomies) due to chromosome segregation errors, the so-called "whole chromosomal" aneuploidy or due to rearrangements of chromosomal parts, often accompanied by deletions, amplifications, or translocations of large regions of the genome that is referred to as a "structural" or "segmental" aneuploidy [3]. Whole chromosomal aneuploidies might arise due to random and sporadic chromosome missegregation events that occur with low frequency during any cell division. The missegregation levels range from 1/1000 to 1/10,000 in human cells [4].

Meiosis generates haploid gametes through a specialized cell division process that consists of one round of DNA replication followed by two cell divisions. The first division, or meiosis I (MI), involves the segregation of homologous chromosomes from each other, whereas meiosis II (MII) involves the segregation of the sister chromatids. Missegregation can also occur in germline cells, and the errors that arise in meiosis result in aneuploid embryos [5]. This chapter aims to provide evidence that supports the use of PGT for embryo evaluation and euploid embryo selection due to a positive correlation with fertilization rates.

2. Incidence of aneuploidy

Errors in meiotic chromosome segregation frequently occur during oogenesis (∼20%), especially during the first meiotic division; this incidence of meiotic errors in oocytes is more elevated in women with advanced maternal age and may be due to the prolonged time that oocytes spend arrested at meiosis I stage, before ovulation [6]. However, some patterns of nondisjunction appear to be chromosome-specific; almost all cases of trisomy 16 are linked to errors at maternal MI, while MII errors are surprisingly common in trisomy 18. Oppositely, in sperm the incidence of aneuploidy is only 2%. Another considerable percentage of errors (∼20%) arise during the first mitosis after fertilization. Among clinically recognized spontaneous abortions (fetal deaths occurring between 6 and 8 weeks and 20 weeks gestation), the incidence increases to ∼50% [7]; the most common specific abnormalities are sex-chromosome monosomy (45,X), accounting for nearly 10% of all spontaneous abortions, and trisomies 16, 21, and 22, which together constitute 50% of all trisomies identified in spontaneous abortions. The incidence among stillbirths (fetal

Chr	Case report	References
1	Pure duplication 1q41-qter: further delineation of trisomy 1q syndromes Partial duplication 1q: reports of five cases and review of the literature	[9] [10, 11]
2	Duplication 2q2.1-q3.1 Partial trisomy 2q: two cases	[12, 13]; [14]
3	Duplication 3p syndrome: reports of three cases and review of the literature	[15–17]
4	Patient with trisomy 4p Partial duplication and duplication region 4q28.3-qter in monozygotic twins with discordant phenotypes	[18] [19, 20]
5	Trisomy 5p: reports of four cases report and review of the literature	[21–23]
6	De novo "pure" partial trisomy (6)(p22.3→pter): case report/review Familial trisomy 6p in mother and daughter	[24] [25]
7	Interstitial de novo tandem duplication of 7 (q31.1-q35) New case of pure partial 7q duplication	[26] [27]

Chr	Case report	References
8	Trisomy 8: report of four cases	[28]
9	Pure 9p trisomy derived from a terminal balanced unreciprocal translocation Trisomy 9: review and report of two new cases	[29] [30]
10	Distal 10q trisomy with copy number gain in chromosome region 10q23.1–10q25.1 Proximal 10q duplication in a child with severe central hypotonia	[31] [32]
11	Partial 11q trisomy syndrome: two cases	[33, 34]
12	Clinical report of a patient with de novo trisomy 12q23.1q24.33	[35]
13	Trisomy 13, Patau's syndrome: reports of three cases	[36–38]
14	Partial proximal trisomy 14	[39]
15	Duplication of distal 15q: reports of 14 cases	[40, 41]
16	Partial trisomy/long arm of chromosome 16: case report/review of literature Complete trisomy 16: a case report	[42] [43]
17	A 790 kb chromosome 17p13.3 microduplication: case report/literature review	[44, 45]
18	Trisomy 18, Edward's syndrome: reports of five cases and discussion	[46–48]
19	Three cases of trisomy 19	[49, 50]
20	20q11.2 duplication syndrome and pure trisomy 20p	[51, 52]
21	Cardiovascular and general health status of adults with trisomy 21	[53]
22	Trisomy 22 syndrome: a report of four cases in newborns and literature review	[54–56]
X	Fragile X syndrome: a case report/review of clinical and molecular diagnoses	[57, 58]
Y	Morphology and pathogenesis of 47,XYY/47,XY patients super male syndrome	[59]

Chr, chromosome number.

Table 1.
Case reports of live births with complete or partial chromosomal abnormalities (mosaic or multiple aberrations are not considered).

deaths occurring between ~20 weeks gestation and term) is ~4% with the types of abnormality being similar to those identified in newborns, and ~0.3% of live-born are aneuploid with the most common abnormalities being trisomies 21, 18, and 13 and sex-chromosome trisomies 47,XXX, 47,XXY, and 47,XYY [5, 8].

2.1 Aneuploidies and live births

Although about 65% of chromosomally abnormal embryos culminate in spontaneous miscarriages, there is still evidence of live births harboring crucial aneuploidies. **Table 1** describes cases that are well documented.

3. Impact of aneuploidy on the efficiency of ART

Assisted reproduction is a solution in many of the growing cases of infertile couples worldwide. A high rate of embryos produced *in vitro* presents chromosomal aneuploidy (~50%), and such aneuploid embryos have reduced the potential for achieving a viable pregnancy. Such abnormalities are recognized as the leading cause of implantation failure and spontaneous miscarriage [60]. Among conceptions that survive to term, aneuploidy is the leading genetic cause of developmental

disabilities and mental retardation [5]. **Table 2** describes data from different infertility centers predominantly showing that aneuploidy rates are similar.

The relatively high aneuploidy rate observed in human embryos after an IVF/ICSI cycle has been attributed to the technique itself since this prevalence seems to be lower in natural conceptions [61]. Many hypotheses have been proposed that may explain these findings: (1) controlled ovarian stimulation treatments, (2) factors related to the ICSI technique and (3) lab conditions as embryo culture.

3.1 Ovarian stimulation and the incidence of embryo aneuploidy

To increase the number of oocytes that can be retrieved for IVF, gonadotrophins are commonly used for superovulation in humans. Exogenous administration of gonadotrophins results in higher concentrations of steroids that may affect oocyte and embryo quality. Ovarian stimulation effects have been well characterized mainly in the murine model and have shown that aggressive stimulation leads to a poorer embryo development potential that could increase the chromosomal abnormality rate [79]. In humans, studies are scarce and less conclusive. A recent study in a population of young normovulatory women showed that a high ovarian response after controlled ovarian stimulation with moderate gonadotropin doses did not increase the embryo aneuploidy rate. Indeed, the higher the ovarian response, the more the euploid embryos obtained [80]; the remaining question is whether this can also be extrapolated to infertile patients with good ovarian reserve.

3.2 Intracytoplasmic sperm injection (ICSI) technique and the incidence of embryo aneuploidy

ICSI has become critical for the treatment of severe male infertility. The principal feature of ICSI is the direct injection of spermatozoa into an oocyte, which facilitates the production of fertilized embryos regardless of semen characteristics, such as sperm concentration and motility. However, the chromosomal integrity of ICSI zygotes is degraded compared to zygotes obtained from an *in vitro* fertilization [81, 82]. During the ICSI procedure, a sperm pretreatment is performed to mimic the conditions of natural fertilization and support the progression of fertilization effects. Studies on mouse models revealed that the chromosomal integrity of zygotes derived from ICSI without any pretreatment of spermatozoa was impaired in comparison with zygotes derived from conventional IVF [83]; even the culture sperm conditions may affect the chromosomal stability of the embryo [84]. Chromosomal damage may occur due to the injection of non-capacitated, acrosome-intact spermatozoa, so to reduce the risk of chromosomal aberrations during the ICSI procedure, it is crucial that sperm capacitation and the acrosome reaction be appropriately artificially induced in the proper medium before use [85].

3.3 Embryo culture and the incidence of embryo aneuploidy

Fertilization and embryo development *in vitro* have the potential to introduce (often inadvertently) stress which cannot only impair embryo development in the laboratory but also have downstream effects after transfer.

In vivo, the developing preimplantation embryo is exposed to gradients of nutrients, hormones, cytokines, and growth factors as it progresses through the fallopian tube to the uterus. Within the lumen of the female tract, the embryo resides in a few 100 nanoliters of a complex viscous fluid characterized by high levels of mucins, albumin, and glycosaminoglycans and by reduced levels of oxygen

N samples (country)	Day of biopsy	Aneuploidy rate	Trisomy %	Monosomy %	Most affected chromosomes	Less affected chromosomes	Ref.
87 (India)	3	54	14.9*	42.5*	22, 18	No data	[62]
150 (Japan)	5	40.6	18*	21.3*	15, 22, 21, 16, 18	4, 12	[63]
52 (UK)	5	40.4	51.3#	48.7#	22, 16, 15, 18, 21, X	1, 2, 5, 10, 17, 19	[64]
12 (UK)	3	75	22*	11*	20, 21, 22	6	[65]
5879 (USA)	3	70.6	No data	No data	No data	No data	[66]
	5	47.8					
759 (UK)	3	64.6	40#	60#	16, 22, 21, 4, 5	4, 6	[67]
274 (US)	3	72.3	39.8*	44.5*	22, 16, 7	6, 9, 19	[68]
192 (Italy)	5	55.2	37.9*	42.7*	No data	No data	[69]
240 (Mexico, Center A)	5	36.3	5.34*	~5*	16, 22, XXX, 9	3, 7, 8, 10, 12, 18, 20	[70]
	6	61.1	5.55*	~10*			
210 (Mexico, Center B)	5	48.9	15.96*	~4*	15, 16, 21, 4	1, 2, 3, 5, 8, 9, 10, 11, 12, 14, 17, 20, 22, X, Y	
	6	43.1	14.65*	~5*			
404 (Mexico)	No data	60.89	No data	No data	4, 15, 22, 16	No data	[71]
15,169 (USA)	No data	No data	No data	No data	13, 15, 16, 18, 19, 21, 22	1, 12, 3	[72]
2204 (UK)	0	74	56#	44#	16, 21, 22, 15, 19	No data	[73]
	3	83	49#	51#	22, 16, 19, 21, 13		
	5	58	47#	53#	22, 16, 15, 21, 19		
21 sets (Italy)	0/1	97.4	Chromosomal loss three times more frequent than gain		22, 15, 16, 17	No data	[74]
	3	47.6+					
	5 or 6	80++					
195 (USA)	0/1	65.5	39.86#	60.14#	22, 13, 15, 16, 19, 21	6, 5, Y, 3	[75]
	5/6	45.2	52#	48#	22, X, 16, 18, 21		
1025 (Mexico)	3/5	43.9	59.3#	40.7#	16, 21, 22, 19, 15, 20	8, 4, 3, 2, 7, 1	Current study

**Percentage of the total number of samples.*
#Percentage of the total number of aneuploid samples.
+Rate from the previous stage of development, PBs to blastomere.
++Rate from the previous stage of development blastomere to TE, PBs = polar bodies, TE = trophectoderm.
For the current study, infertile patients who underwent ART at the Ingenes Institute were included. The patients were clinically evaluated according to a standardized protocol that includes family and personal clinical history. The protocol was approved by the Ethics Committee of the Ingenes Institute, and a signed informed consent was obtained from all patients. IVF, embryo biopsy, and mCGH were performed according to the standard protocols of the Institute Ingenes as previously described [76, 77]. Only optimal morphological embryos were considered for this study. Selection and embryo transfer were done on Day 3 or Day 5 of development according to the embryo morphological assessment, using the criteria established by the Istanbul consensus Workshop on Embryo Assessment [78].

Table 2.
Aneuploidy rates of different IVF clinics around the world; when mentioned, the most commonly affected chromosomes are listed.

(typically 2–8%). The embryo is in constant motion, moved by gentle ciliated and muscular action of the female tract [86]. This scenario is in stark contrast to the laboratory environment, where typical gametes and embryos are exposed to relatively large volumes of culture medium, remain static during culture while resting on a polystyrene substrate, and create unstirred layers where the end products of metabolism concentrate and nutrients become limited [87].

Embryos are sensitive to both chemical and physical signals within their microenvironment. Factors within the laboratory as oxygen level, ammonium released from amino acids into the culture, poor laboratory air quality, temperature and pH, oil overlay, embryo culture volume/density, the static nature of culture, light, or even mechanical factors as pipetting, can negatively impact gametes and embryos and generate stress. When more than one stress factor is present in the laboratory, more negative synergies can result, and these factors play a significant role in influencing the development and events post transfer [88]. For example, recent studies have reported that a decrease in temperature has the potential to affect the stability of the oocyte's meiotic spindle, reducing fertilization rates, delaying embryo development, and decreasing clinical pregnancy rates [89]. However, more studies are needed to demonstrate the impact of embryo culture on aneuploidy rates.

4. Aneuploidy detection: techniques for PGT

PGT is the genetic diagnosis analysis performed to identify euploid embryos before uterine transfer [90]. PGT determines the numeric chromosomal constitution of a cellular biopsy sample obtained from a cultured embryo to determine its competence [91, 92].

PGT was first described in 1990 by Handyside et al. [93] when the sex of the six- to eight-cell stage embryos from two couples with a known risk of transmitting X-linked diseases was assessed by DNA amplification of a Y chromosome-specific repeat sequence. The earliest PGT studies in the 2000s were based on the fluorescence in situ hybridization (FISH) technique where 3–12 chromosomes can be analyzed on the cleavage stage or polar body biopsies [90]. Those studies had disappointing results in clinical practice since it had no beneficial effect on live birth rate after IVF [94]. The major drawback of FISH-based PGT is the limited number of chromosomes that can be analyzed considering that aneuploidy can affect any of the 22 autosomes and both sex chromosomes [95]; consequently, there have been dramatic improvements in PGT technology to make it valuable for clinical practice.

Nowadays, several methodologies for 24-chromosome analysis are available for clinical use that aim to increase implantation rates and decrease miscarriage rates associated with IVF [90]: microarray comparative genomic hybridization (mCGH), single-nucleotide polymorphism (SNP) microarray, real-time polymerase chain reaction (qPCR), and next-generation sequencing (NGS) [96, 97]. This review will focus on the relevant aspects of the PGT techniques used in our laboratory.

4.1 Microarray comparative genomic hybridization (mCGH)

mCGH is a ratio labeling protocol to compare the DNA product of a clinical sample to a healthy control. For PGT, biopsied embryonic cells must be lysed to extract the sample's DNA, which will be further amplified by a protocol that provides whole genome coverage [90, 95, 98]. The resulting DNA products are co-hybridized with a standard DNA control sample (46,XY and 46,XX) with a series of site-specific fluorophores on a microarray chip with approximately 4000 markers spaced throughout the genome [90]. Then, a confocal laser platform

detects the relative color intensity, and a bioinformatics compares the intensity of each fluorophore in the sample versus the control to identify any bias and determine the ploidy status of the sample [90, 95, 98].

The mCGH analysis reports the ratio of sample DNA to a reference DNA, as a chromosomic profile where the molecular karyotype is represented. Usually, the sample DNA is labeled with a green fluorescent dye, while the reference DNA sample is tagged red [99]. Thus, diploid embryos will have a relatively equal ratio of green-to-red fluorescence in every pair of chromosomes, represented as a continuous horizontal plot line. Monosomy will be represented as a clear downward deviation in the plotted line, indicating a relative lack of green-to-red signal intensity; in contrary, a trisomy will be displayed as an upward deviation in the plotted line due to a relative increase in the green-to-red signal intensity.

The specificity rate of mCGH-based PGT is about 99% [90]. The test results can be available within 12–15 h, considering that the entire analysis can be performed during this short time frame [90, 91]. Additionally, brand-specific features are offered by each manufacturer: Agilent's GenetiSure Pre-Screen Microarray offers a detection rate of 100% for aberrations >10 Mb and 89% for >5.3 Mb [100]; KaryoLite BoBs Kit from Perkin Elmer uses an alternative BACs-on-Beads technology and results are interpreted by the BoBsoft™ analysis software [99]; and RHS's EmbryoCellect Kit is the only mCGH-based PGT validated for mosaicism detection [101]. Recently, Illumina's 24sure PGS Microarray had been discontinued, and the NGS-based VeriSeq PGS is now offered as an alternative solution [102].

mCGH entails some disadvantages: first, the embryo sample requires a previous whole genome amplification (WGA) process to support single-cell diagnostics by mCGH [95], raising the possibility of introducing errors during the amplification [91]; second, mCGH is a semiquantitative technique that only reports the ratio of sample DNA to a reference DNA; it is to say that only imbalances in DNA content can be identified. Therefore, mCGH is unable to detect uniparental disomic or triploid embryos as it cannot discriminate between 46,XX from 69,XXX, and 46, XY from 69,XXY [90, 91, 95]. Last, the mCGH used for PGS cannot identify structural chromosome aberrations or diagnose mosaicism in a trophectoderm sample [90].

4.2 Next-generation sequencing (NGS)

NGS refers to the emerging technology of non-Sanger-based DNA sequencing that allows the sequence in parallel millions of DNA strands with high-throughput yield. In the field of ART, this powerful tool is being applied for PGT to replace cytogenetic microarrays [98, 102].

Different platforms are commercially available for NGS with different technological approaches. Illumina's MiSeq NGS platform applies a sequencing-by-synthesis method, where DNA is attached and amplified in situ to be subsequently used as a template for synthetic sequencing with fluorescent-labeled reversible-terminator nucleotides [103]. Ion Torrent NGS technology, commercialized by ThermoFisher Scientific, is based on collecting data by sensing the hydrogen ions that are released as by-products when nucleotides are incorporated by a template-directed DNA polymerase synthesis on an ion chip [104].

Despite the dissimilarities between platforms, the common basis of chromosome copy number analysis by NGS is the fragmentation of the amplified DNA sample into small segments of 100–200 base pairs that are further sequenced in parallel until the number of reads covering a determined position in the genome is attained, in general, a 30× coverage (sequencing each base pair 30 times) ensures sufficient accuracy. The sequence data obtained are then compared with a reference genome

and counted by bioinformatics software. The copy number of a specific chromosome should be proportional to the number of counted sequences; therefore, an increase or reduction in the number of reads will, respectively, represent a trisomy or monosomy [97, 99].

NGS allows to simultaneously perform both qualitative and quantitative analyses of multiple embryos with high-resolution data for chromosomal analysis [96, 97]. The higher sensitivity and precision offered by NGS [96, 105, 106] makes possible to exclude embryos with mosaicism [105, 106] and partial aneuploidies or triploidies [106], improving pregnancy outcomes due to its enhanced capability for detecting those challenging abnormalities.

PGT by NGS can predict not only chromosome copy number for the diagnosis of whole chromosome aneuploidy with 99.98% assignment consistency [97] but also single-gene disorders [107], abnormalities of the mitochondrial genome [108], and segmental chromosome imbalances [97, 99]. Balanced chromosomal rearrangements cannot be detected by NGS [97].

The increasing demand and accelerated development are continuously reducing the cost of NGS technology [109]. Also, potential cost-benefit ratios can be achieved when the full sequencing capacity of the apparatus is exploited [96, 97, 99]. Furthermore, molecular tools, like barcoding, are being implemented to allow multiplex high-throughput sequencing [110]; this promising strategy will reduce the diagnosis' cost per patient by performing simultaneous analysis of multiple embryos from different patients [97].

5. Aneuploidy and women age

In our study, by analyzing the mCGH data, the total number of aneuploidies was found to be 734, and from these, 641 (87.3%) were derived from patients and 93 (12.7%) from donors. Overall, this study displayed similar rates of monosomies, trisomies, double aneuploidies, and multiple aneuploidies. The total number of monosomies (191) was similar to the number of trisomies (194), accounting for 26 and 26.4% of the total aneuploidies, correspondingly. Furthermore, the total number of double (165) and multiple (184) aneuploidies was also very similar, accounting for 22.5 and 25.1% of the total aneuploidies, correspondingly. Nevertheless, it is worth noticing that when considering only the donor group, monosomies seem to be more prevalent: 38.7% of the total donors' aneuploidies were monosomies vs. 24.7% of trisomies, 16.1% of double aneuploidies, and 20.4% of multiple aneuploidies; what is more, the percentage of monosomies in the donor group is higher than that of the monosomies of the patient group (38.7 vs. 24.3%). The most common monosomies affected chromosomes 15, 16, and 22, whereas the most common trisomy affected chromosomes 16, 19, and 21 (**Table 3**).

It has been shown that the lowest risk for embryonic aneuploidy is between ages 26 and 30, with aneuploidy rates steadily increasing with maternal age after 26 years of age [111] and leaping significantly from the age of 39 [112]. For this reason, women of advanced maternal age are encouraged to favor oocyte donation to yield high-quality viable embryos.

Interestingly, some studies have identified that women of younger ages possess an increased prevalence of aneuploidy, with >40% of aneuploidy in women of 23 years and under [111] and 58% of aneuploidy in women of <31 years of age. In the current study, both the donor (≤29 years) and the patient group of ≤29 years

	All	Patients	Donors
Total	734	641	93
Monosomy	191 (26.0%)	155 (24.3%)	36 (38.7%)
−Chr 15	13	13	0
−Chr 16	21	18	3
−Chr 22	22	22	0
−Chr X	10	8	2
−Chr Y	40	26	14
Trisomy	194 (26.4%)	171 (26.7%)	23 (24.7%)
+Chr 16	26	24	2
+Chr 18	9	7	2
+Chr 19	23	21	2
+Chr 20	17	13	4
+Chr 21	20	20	0
+Chr 22	19	19	0
+Chr X	10	8	2
+Chr Y	1	1	0
Dual	165 (22.5%)	150 (23.4%)	15 (16.1%)
Multiple	184 (25.1%)[a]	165 (25.7%)[b]	19 (20.4%)[c]

The current study included 441 patients, resulting in 474 cycles. A total of 1629 embryos were analyzed; from those, 54 were excluded due to failed WGA, leaving 1575 embryos for analysis, 1258 from patients, and 317 from donors. Biopsies were performed at the blastomere (Day 3, patients = 238 and donors = 50) and blastocyst stages (Day 5, patients = 1020 and donors = 267). Finally, 734 embryos (46.6%) were found to be aneuploid (patients = 641 and donors = 93). The total number of monosomies and trisomies is provided along with their respective percentages; furthermore, the number of the most common aneuploidies of the mCGH data is listed.
[a]*Nine embryos had completely abnormal mCGH profiles.*
[b]*Even embryos had completely abnormal mCGH profiles.*
[c]*Two embryos had completely abnormal mCGH profiles.*

Table 3.
Most frequent types of aneuploidies in the mCGH data of the current study.

displayed high aneuploid rates, 28.5 and 27.4%, respectively (**Table 4**). Given the high rates of aneuploidy in younger women, attention should be paid in detecting aneuploidy in embryos from women of young maternal age, especially since this group of patients is not routinely encouraged to perform a PGT. Still, whether there is a difference between the distribution of aneuploidies between donors and patients remains uncertain.

When stratifying our analysis in age groups (a, ≤29; b, 30–34; c, 35–37; d, 38–40; e, 41–43; and f, ≥44 years of age), a visible continuous increase in aneuploidy rate can be observed as maternal age increases (**Table 4**); furthermore, this increase in aneuploidy goes hand in hand with a continuous decrease in implantation, as it can be observed in the decrease of positive beta-human chorionic gonadotropin (β-hCG) values as age increases (β-hCG values ≥10 mUI/ml from Day 14 after transference were considered positive).

Category	≤29 a	30–34 b	35–37 c	38–40 d	41–43 e	≥44 f	Donors g
Number of samples (n, cycles)	27	66	72	105	90	31	83
Age (years)	$27.0 \pm 2.4^{b,c,d,e,f,g}$	$32.5 \pm 1.3^{a,c,d,e,f,g}$	$36.1 \pm 0.7^{a,b,d,e,f,g}$	$39.0 \pm 0.8^{a,b,c,e,f,g}$	$41.8 \pm 0.8^{a,b,c,d,f,g}$	$45.0 \pm 1.6^{a,b,c,d,e,g}$	$22.8 \pm 3.0^{a,b,c,d,e,f}$
Body mass index (kg/m^2)	23.6 ± 3.8^{g}	24.6 ± 3.8^{g}	24.3 ± 3.9^{g}	24.4 ± 3.7^{g}	24.8 ± 3.8^{g}	24.6 ± 2.8^{g}	$21.8 \pm 2.5^{a,b,c,d,e,f}$
Ova collected (n)	$16.8 \pm 7.9^{e,f}$	$17.3 \pm 9.8^{d,e,f}$	$16.1 \pm 9.0^{d,e,f}$	$12.0 \pm 6.3^{b,c,g}$	$11.0 \pm 5.9^{a,b,c,g}$	$8.9 \pm 6.0^{a,b,c,g}$	$15.8 \pm 8.1^{d,e,f}$
Ova fertilized (n)	$14.3 \pm 6.9^{e,f}$	$15.2 \pm 8.6^{d,e,f}$	$13.6 \pm 7.5^{d,e,f}$	$10.3 \pm 5.2^{b,c,g}$	$9.8 \pm 5.5^{a,b,c,g}$	$7.6 \pm 4.8^{a,b,c,g}$	$13.9 \pm 6.2^{d,e,f}$
Embryos (n)	$11.2 \pm 6.3^{e,f}$	$11.7 \pm 7.9^{d,e,f}$	$10.6 \pm 6.0^{d,e,f}$	$7.7 \pm 4.2^{b,c,g}$	$7.5 \pm 4.6^{a,b,c,g}$	$6.2 \pm 3.9^{a,b,c,g}$	$10.4 \pm 4.6^{d,e,f}$
Fertilization rate (%)	77.9 ± 17.0	76.3 ± 15.1	79.7 ± 14.2	76.6 ± 16.6	77.0 ± 18.9	83.6 ± 15.2	76.1 ± 16.0
Aneuploidy rate (%)	$27.4 \pm 33.3^{d,e,f}$	$36.0 \pm 29.2^{d,e,f}$	$35.7 \pm 30.3^{d,e,f}$	$57.7 \pm 34.9^{a,b,c,f,g}$	$66.6 \pm 37.7^{a,b,c,g}$	$87.4 \pm 19.5^{a,b,c,d,g}$	$28.5 \pm 28.7^{d,e,f}$
Pregnancy rate (%)	$60.87^{b,c,d,e,f,g}$	$50.99^{a,c,d,e,f,g}$	$49.09^{a,b,d,e,f,g}$	$44.00^{a,b,c,e,f,g}$	$55.17^{a,b,c,d,f,g}$	$42.86^{a,b,c,d,e,g}$	$50.79^{a,b,c,d,e,f}$

Values are shown as mean ± standard error. Significance was determined by one-way ANOVA followed by a Bonferroni or Dunnett's T3 post hoc *test. Superscripts indicate a significant difference ($p < 0.05$, two-tailed):*
[a]*Versus 29 years old group.*
[b]*Versus 30–34 years old group.*
[c]*Versus 35–37 years old group.*
[d]*Versus 38-40 years old group.*
[e]*Versus 41–43 years old group.*
[f]*Versus 44 years old group.*
[g]*Versus donors.*

Table 4.
Comparative of aneuploidy and pregnancy rates between age groups.

6. Remarks

One of the most critical reasons for unsuccessful IVF procedures is implantation failure due to aneuploid embryos. Aneuploidies are the primary cause of perinatal death and genetic abnormalities; consequently, the detection of chromosomal disorders constitutes the most frequent indication for PGT. Here, we report on the aneuploidy rates found in IVF procedures in Mexico. Even though there are studies that assert that PGT does not improve pregnancy rates, we show that aneuploidy rates inversely correlate with implantation and that levels of aneuploidy among high morphological quality embryos are still an important issue to be faced in everyday ART practice, and this evidence works in favor of continuing to use PGT analysis.

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Abbreviations

ANOVA	analysis of variance
ART	assisted reproductive techniques
Chr	chromosome
FISH	fluorescence in situ hybridization
ICSI	intracytoplasmic sperm injection
IVF	*in vitro* fertilization
mCGH	microarray comparative genomic hybridization
MI	meiosis I
MII	meiosis II
NGS	next-generation sequencing
OR	odds ratio
PGS	preimplantation genetic screening
PGT	preimplantation genetic testing
qPCR	real-time polymerase chain reaction
SNP	single-nucleotide polymorphism
WGA	whole genome amplification
β-hCG	beta-human chorionic gonadotropin

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