

Paternal advanced age and sperm quality are indicators for Preimplantation Genetic Testing in egg donation cycles

Jeimy Pedraza¹, Hugo Sierra¹, Jacqueline Cordero¹, Esther López-Bayghen^{2,*}

¹Laboratorio de Fertilización in Vitro, Instituto de Infertilidad y Genética México SC, Ingenes, 05320 México City, México

²Departamento de Toxicología, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), 07360 México City, México

*Correspondence: ebayghen@cinvestav.mx (Esther López-Bayghen)

DOI: [10.31083/j.ceog4806222](https://doi.org/10.31083/j.ceog4806222)

This is an open access article under the CC BY 4.0 license (<https://creativecommons.org/licenses/by/4.0/>).

Submitted: 17 March 2021 Revised: 20 April 2021 Accepted: 8 June 2021 Published: 15 December 2021

Background: A wide variety of studies demonstrate the influence of the oocyte source's age on the success of assisted reproduction treatments; however, the effect of paternal age has been studied to a lesser extent. Therefore, our goal was to assess the impact of male age and sperm quality on *in vitro* fertilization (IVF) outcomes. **Methods:** Three hundred ninety-four ova donation IVF cycles from Ingenes México were retrospectively analyzed. All ova donors (age range: 18–35 years) underwent a similar IVF stimulation protocol. The oocytes were aspirated and inseminated by intracytoplasmic sperm injection (ICSI) using either partner sperm ($n = 332$, age: 42.4 ± 7.4 years) or donor sperm ($n = 62$, age: 25.0 ± 3.3 years). Semen characteristics were evaluated by a seminogram. Biopsies were performed on Day 5 or Day 6, and Preimplantation Genetic Testing for Aneuploidies (PGT-A) was used to determine chromosome integrity with Next-Generation Sequencing. **Results:** A total of 1449 embryos were biopsied, 995 Day 5 and 454 Day 6. The aneuploidy rates for Day 5 and Day 6 embryos were not significantly different (22.2% and 22.7%, respectively). Independent of the sperm source, there was no observable trend between embryo aneuploidy rates and the sperm source's age; however, when considering paternal sperm with teratozoospermia, a significant association was observed ($\rho = 0.165$, $p = 0.041$). Sperm quality showed no effect on fertilization rates, blastocyst formation, or implantation rates. **Discussion:** Here, the sperm source's age did not affect the aneuploidy rate nor embryo implantation; however, with teratozoospermia, there was a weak association between the aneuploidy rate and age. Therefore, it would be prudent to perform PGT-A when advanced age and teratozoospermia are detected.

Keywords

Aneuploidy; Egg donor; *In vitro* fertilization; Paternal age; Preimplantation genetic screening

1. Introduction

Male partner's effects are usually considered negligible during IVF, even though over 40 to 50% of infertility diagnoses are associated with male factor [1, 2]. An increase in paternal age and age-independent degeneration of semen quality can be associated with a decline in viable embryos during IVF [3]. Numerous studies have demonstrated that advanced maternal age is associated with decreased ovarian

reserve, oocyte pick-up, fertilization rates, and the production of high-quality embryos [4–6]. However, concerning paternal age, few studies have examined this association, with some studies demonstrating a significant association between paternal age and the embryo aneuploidy rate [7–9] as well as deterioration of sperm concentration, motility, and morphology [10–14]. Therefore, it has been suggested that, under certain circumstances, such as low sperm quality in the presence of advanced paternal age, a couple should consider using donor sperm and PGT-A [15]. This begs the question, when semen quality is considered, does using donor sperm improve IVF outcomes? Furthermore, if the male factor is considered, is this an indication to perform PGT-A? Therefore, this study aimed to compare the embryo aneuploidy rates as well as the implantation and pregnancy rates between paternal sperm and donor sperm, with respect to the sperm source's age and sperm abnormalities.

2. Methods

2.1 Study design

We performed a clinical retrospective study by reviewing all medical records from October 2016 to December 2018 at Ingenes México, México City, México. To be included in the study, the infertile patients had to undergo IVF using oocyte donation, and the embryos had to be assessed by PGT-A using Next-Generation Sequencing (NGS). As exclusion criteria, the oocyte recipients were not diabetic or obese, taking medication for high blood pressure or any other medications that would affect IVF. For their male partners, the subjects also had not to be suffering from diabetes, obesity or taking medications (antibiotics and retroviral agents) that would affect the production or quality of their sperm. PGT-A was performed only on embryos with sufficient morphological quality to survive the biopsy and freezing procedures.

2.2 Oocyte donors

All oocyte donors were between 18 and 35 years old, had normal-appearing ovaries confirmed by transvaginal ultrasound, an antral follicle count >8 , and displayed a correct

response to ovarian stimulation through a progressive and gradual increase in estradiol levels and follicular sizes, concordant with FSH administration. A standard course of controlled ovarian stimulation was performed in a step-down protocol with a GnRH antagonist daily dose (Cetrotide 0.25, Merck, Darmstadt, Germany). Follicular stimulation was performed with menotropin daily administration (mixture of urinary FSH and LH, Ferring Laboratories, Saint-Prex, Switzerland), and the doses were calculated depending on the donor's response. The stimulation was prolonged until the diameter of the leading follicles was >18 mm. Afterward, the trigger was performed only with Gonapeptyl Daily (Ferring Pharmaceuticals, Saint Prex, Switzerland) (triptorelin/acetate) to avoid hyperstimulation. Oocyte collection was performed 36 h after triggering by means of ultrasound-guided transvaginal follicular aspiration. All 14–18 mm follicles were aspirated, and the ova were collected.

2.3 Semen collection and sperm assessment

Sperm donors were between 18 and 35 years old, and their samples were only used after being frozen and passed a 6-month quarantine at the Donor Sperm Bank at Ingenes Institute in México City. All donor sperm were diagnosed as normozoospermic (exceeding the minimum acceptable value for each category as determined by the World Health Organization criteria for the Examination and Processing of Human Semen) before cryopreservation [16]. All paternal sperm samples were fresh (not frozen) and ranged in quality. Independent of the sperm source (paternal or donor), before the semen sample was collected, the patient or donor was explained to abstain from sexual activity and drug use for 3 to 5 days. The semen was obtained by masturbation, and in the case of azoospermia, the sample was obtained by Percutaneous Epididymal Sperm Aspiration. All sperm samples were analyzed for volume, concentration, progressive motility, and morphology by phase-contrast microscopy. For concentration and motility, the counting was performed in a Makler Chamber® (Sefi-Medical Instruments, Haifa, Israel) with 20x magnification. The morphological assessment was performed by stained smearing with 100x magnification. The limit values applied for diagnosis in volume, concentration, and motility were according to the World Health Organization 2010 criteria [16]. For morphology, the Kruger criteria were used [17]. Sample preparation was performed according to standard laboratory procedures (washing, density gradients, and swim-up), and diagnosis and sperm capacitation were performed using the World Health Organization criteria [16].

2.4 Embryo fertilization, culture, and biopsy

The oocytes were all inseminated by ICSI, and fertilization was judged by forming two pronuclei and two polar bodies, 16–18 h after insemination. Embryos were cultured (37 °C, 7.5% CO₂) in a single-step system using Global Total L.P. media (Cat H5gt-030, Life Global, CooperSurgical Fertility Solutions, Ballerup, Denmark) from Day 1 to either Day 5 or

Day 6. Once the embryos reached the blastocyst stage, the embryos' morphology was assessed using the criteria according to Gardner and Schoolcraft [18]. Embryos selected for biopsy were those that reached the expanded blastocyst stage on Day 5 or Day 6 and had good morphological quality according to the inner cell mass and trophectoderm evaluation. The embryos were placed in human tubal fluid (HTF)-HEPES media supplemented with 10% HSA (Cat 2001, Invitro Care & GHSA-125, Life Global, CooperSurgical Fertility Solutions, Ballerup, Denmark). Trophectoderm cells were isolated using a Hamilton Thorne Zilos laser (ZILOS-tk®, Hamilton Thorne, Beverly, MA, USA) to remove the zona pellucida and collected using biopsy micropipettes (MBB-FP-SM-30, Origio, Ballerup, Denmark). A total of 4 to 7 cells were retrieved, washed, and placed into a 0.2-μL PCR tube. Immediately after the biopsy, the embryos were frozen by vitrification [19].

2.5 Genomic testing

For PGT-A, we used NGS. According to the manufacturer's instructions, each biopsy sample was amplified using the SurePlex DNA amplification system (Illumina Inc., San Diego, CA, USA). Whole-genome amplification (WGA) products were quantified using a Qubit 3.0 Fluorometer (Life Technologies, Carlsbad, CA, USA). Library preparation was carried out with the VeriSeq PGS Library Prep Kit (Illumina Inc.). DNA 'indexing' was performed to simultaneously analyze samples from different embryos using the Nextera XT 96-Index Kit (Illumina Inc.). For library preparation, 5 μL (0.2 ng/μL) of each WGA product from each sample was tagged (tagged and fragmented) by the VeriSeq PGS transposome, using the manufacturer's protocol, and neutralized by adding 5 μL of neutralization buffer. The tagged DNA was amplified with index one primers (N701 and N712, Illumina Inc.) and index two primers (S503 and S504, Illumina Inc.) to become the NGS library via a limited cycle PCR program. Each sample's NGS library was purified to remove short fragments and primers. Finally, NGS libraries were pooled, denatured with HT1, and loaded into a VeriSeq PGS (Illumina Inc.) sequencing cartridge following the manufacturer's protocol. NGS library was sequenced with a MiSeq apparatus and the MiSeq Reporter Software (Illumina Inc.).

2.6 Embryo recipients and transfer

Oocyte donation indications were failed IVF cycles with own oocytes, low ovarian reserve, poor oocyte quality, genetic or chromosomal abnormalities transmissible to offspring, and spontaneous/iatrogenic menopause. Before the thawed embryos were transferred, estrogen endometrial preparation was performed using an increasing transdermal dose of estradiol valerate for a period of 15 to 40 days before the start of luteal support with vaginal progesterone for five days. Embryos were transferred 2 hours after being thawed. The Reproductive Specialist/Embryologist determined clinical decisions about which and how many embryos should be transferred. Depending on the embryo survival rate and specific clinical conditions related to each case, every deci-

Table 1. Characteristics of the study participants.

Category	Total	Donor semen		Paternal semen		p-value
		Normozoospermia	Normozoospermia	Teratozoospermia	Severe alterations	
IVF cycles	394	62	78	153	101	
Embryos analyzed	1447	227	297	558	365	
Paternal Age	39.7 ± 1.0	25.0 ± 3.3 ^{b,c,d}	40.3 ± 7.2 ^{a,d}	42.0 ± 6.5 ^{a,d}	44.7 ± 8.2 ^{a,b,c}	<0.001*
Maternal age						
Donor	24.5 ± 3.5	24.7 ± 3.2	24.9 ± 3.6	24.4 ± 3.4	24.1 ± 3.7	0.467
Recipient	41.7 ± 4.1	41.7 ± 3.1	40.5 ± 4.6 ^{c,d}	42.1 ± 4.3 ^b	42.1 ± 3.9 ^b	0.031*
Biopsy Day						
Day 5	307 (77.9 ± 2.2%)	51 (82.3 ± 4.8%)	64 (82.1 ± 4.6%)	119 (77.8 ± 3.3%)	73 (72.3 ± 4.4%)	0.344
Day 6	87 (22.1 ± 2.2%)	11 (17.7 ± 4.8%)	14 (17.9 ± 4.6%)	34 (22.2 ± 3.3%)	28 (27.7 ± 4.4%)	
Male etiology						
Normozoospermia	140 (35.5 ± 2.3%)	100.0%	100.0%	-	-	
Teratozoospermia	153 (38.8 ± 2.5%)	-	-	100.0%	-	
Asthenoteratozoospermia	37 (9.4 ± 1.5%)	-	-	-	37 (36.6 ± 4.8%)	
Asthenozoospermia	19 (4.8 ± 1.1%)	-	-	-	19 (18.8 ± 3.8%)	
Azoospermia	6 (1.5 ± 0.6%)	-	-	-	6 (5.9 ± 2.3%)	
Cryptozoospermia	2 (0.5 ± 0.3%)	-	-	-	2 (2.0 ± 1.4%)	
Oligoasthenoteratozoospermia	21 (5.3 ± 1.1%)	-	-	-	21 (20.8 ± 4.1%)	
Oligoteratozoospermia	16 (4.1 ± 1.0%)	-	-	-	16 (15.8 ± 3.6%)	

Results are presented as frequency, percent and standard error, or mean and standard deviation. Differences between groups were calculated using either the Chi-Square test for categorical data, an ANOVA with a *post hoc* Bonferroni test for parametric, continuous data, or the Kruskal-Wallis test with a *post hoc* Dunn's test for non-parametric, continuous data. A significant difference between the groups ($p < 0.05$) are indicated by an asterisk (*).

^a vs. Donor normozoospermia sperm, $p < 0.05$.

^b vs. Paternal normozoospermia sperm, $p < 0.05$.

^c vs. Paternal sperm with teratozoospermia, $p < 0.05$.

^d vs. Paternal with severe sperm alterations, $p < 0.05$.

sion was made with the patient's approval. Clinic pregnancy was considered positive if the β -hCG serum levels were >10 mUI/mL 14 days after embryo transfer. The embryo implantation was confirmed using ultrasound by detecting a gestational sac with a fetal heartbeat at 6 to 8 weeks.

2.7 Sample size calculation

To determine the required sample size, we used the G*Power 3 software (<https://stats.idre.ucla.edu/other/gpower/power-analysis-for-one-sample-t-test/>). For the comparison between normal paternal and donor semen, using the assumptions that the expected aneuploidy rate is around 20% of ova donor cycles with a standard deviation of 15% and that a clinical difference of 10% would indicate a significant result, $\alpha = 0.05$, $\beta = 0.05$, and a one-to-one ratio, we determine that 60 IVF cycles would be required for each group.

2.8 Statistical analysis

All analyses were carried out with the Statistical Package for the Social Sciences software v26.0 (IBM, Chicago, IL, USA). The Shapiro-Wilk test assessed the normality of the data. Differences between categorical data were assessed with the Chi-Square test. Homogeneity of the variances in parametric data was determined with Levene's test. For parametric data, differences between groups were determined with

Analysis of Variance (ANOVA) with a *post hoc* Bonferroni test. For non-parametric data, differences between groups were determined with the Kruskal-Wallis test with a *post hoc* Dunn's test. Pearson correlation coefficient (r) or Spearman correlation coefficient [6] were used to determining the association between variables. p -values < 0.05 (two-tailed) were considered statistically significant.

3. Results

We originally identified 457 ova donor cycles. However, using the inclusion and exclusion criteria, 394 IVF cohorts qualified for the study (Fig. 1). As shown in Table 1, 15.7% (95% CI: 12.2–19.8%) were from donor sperm, 19.8% (95% CI: 16.0–23.9%) were from parental sperm with normal seminal parameters (normozoospermic patients), 38.8% (95% CI: 33.8–43.7%) were from parental sperm that suffered from teratozoospermia, whereas 25.6% (95% CI: 21.6–29.9%) were from parental sperm that suffered from severe alterations in one or more seminal parameters (volume, concentration, progressive motility, and morphology). There was a 17-year difference between the average age of partner sperm (42.4 ± 7.4 years) or donor sperm (25.0 ± 3.3 years), which is considered small. Even though there was a statistical difference in the ages between the group with paternal sperm with

no alterations and the paternal sperm with alterations, this was not clinically different. As expected, the sperm donors were significantly younger. There were no differences between the study groups concerning the ova donors' ages, and there was no clinical difference between the maternal recipients' ages. Lastly, the average aneuploidy rates for the study groups (Donor semen normozoospermic: 15.9%, 95% CI: 10.0–21.9%; Patient semen normozoospermic: 22.4%, 95% CI: 16.7–28.2%; Patient semen with teratozoospermia: 17.5%, 95% CI: 13.2–21.7%; and Patient semen with severe abnormalities: 21.3%, 95% CI: 16.4–26.2%) were not significantly different (Fig. 2); moreover, the type of male etiology did not affect the aneuploidy rates (Supplementary Fig. 1). There were no differences between the groups with respect to the type of aneuploidies (monosomies, trisomies, both, and multiple changes) as well as the specific chromosome loss or gain (Supplementary Tables 1,2).

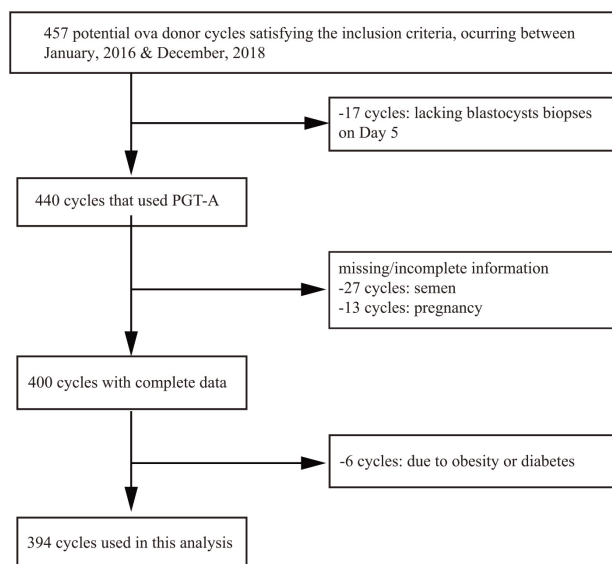


Fig. 1. The study flow chart in line with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement.

For patient sperm, there was a weak correlation between paternal age and the prevalence of semen abnormalities (Pearson's $r_{bivariate} = 0.165$, $p = 0.003$, data not shown). When the study group was stratified by paternal age into quartiles, there was no significant trend for the donor group (Fig. 3A). For the paternal sperm source, there was also no observable association between the sperm source age and the embryo aneuploidy rate (Fig. 3B). However, when stratified into normozoospermia (Fig. 3C), teratozoospermia (Fig. 3D), and severe sperm abnormalities (Fig. 3E), only the teratozoospermia group presented with an age-dependent association (Spearman $\rho = 0.165$, $p = 0.041$).

When the IVF outcomes were compared, there was no clinical difference between the study groups with respect to

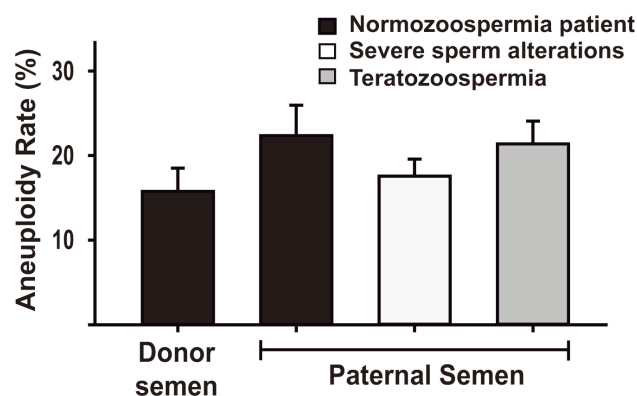


Fig. 2. Aneuploidy rates for embryos developed from donor ova using patient or donor sperm, stratified into normozoospermic donor (left black column), normozoospermic patient (right black column), patients with teratozoospermia (white column), and patients with severe abnormalities (gray column). The column height corresponds to the average aneuploidy rate, whereas the bar length is the standard error. Differences between groups were determined with ANOVA with a *post hoc* Bonferroni test. Significant differences ($p < 0.05$, two-tailed) are indicated with an asterisk.

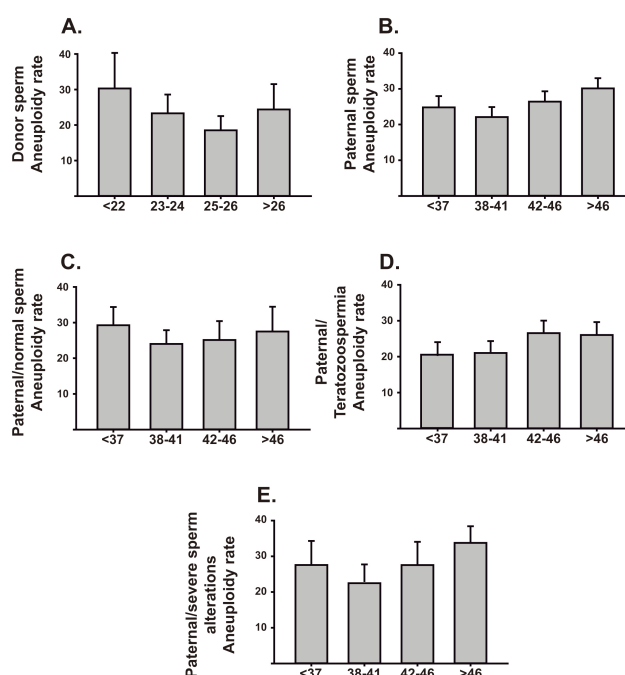


Fig. 3. Aneuploidy rates for embryos developed from donor ova using patient or donor sperm, stratified by age. Data were separated into quartiles based on the sources' age of the donor sperm (A) or paternal sperm (B). Afterward, the paternal sperm was categorized, based on etiology, as either normozoospermic (C), teratozoospermic (D), or severe abnormalities (E). The column height corresponds to the average aneuploidy rate, whereas the bar length is the standard error. Associations between the sources' age and the aneuploidy rate were determined by calculating Spearman's ρ .

the number of ova captured, the number of ova fertilized, the number of blastocysts formed, or the embryo transfer rate

Table 2. The effects of the source of sperm on IVF outcomes.

Category	Total	Donor semen		Paternal semen		p-value
		Normozoospermia	Normozoospermia	Teratozoospermia	Severe alterations	
Ova captured (n)	14.9 ± 4.7	13.1 ± 5.9 ^{b,c}	15.4 ± 4.5 ^a	15.4 ± 3.7 ^a	14.9 ± 4.9	0.039*
Ova Fertilized (n)	10.0 ± 3.3	9.0 ± 4.1	10.3 ± 3.0	10.1 ± 2.9	10.0 ± 3.5	0.198
Blastocyst formed (n)	5.7 ± 2.5	5.6 ± 2.5	5.9 ± 2.3	5.7 ± 2.4	5.5 ± 2.9	0.703
Embryos Transferred (n)	1.9 ± 0.6	2.0 ± 0.6	1.9 ± 0.5	1.9 ± 0.6	1.8 ± 0.6	0.176
Transfer rate (%)	44.0 ± 39.3	41.1 ± 37.9	50.0 ± 40.1	40.4 ± 37.2	47.0 ± 42.9	0.299
No transferred	32 (8.1 ± 1.4%)	5 (8.1 ± 3.6%)	4 (5.1 ± 2.4%)	9 (5.9 ± 1.8%)	14 (13.9 ± 3.4%)	
β-hCG results (n)						0.746
Negative	132 (36.5 ± 2.5%)	22 (38.6 ± 6.3%)	23 (31.1 ± 5.4%)	55 (38.2 ± 4.1%)	32 (36.8 ± 5.3%)	
Positive	230 (63.5 ± 2.5%)	35 (61.4 ± 6.3%)	51 (68.9 ± 5.4%)	89 (61.8 ± 4.1%)	55 (63.2 ± 5.3%)	
Ultrasound results (n)						0.711
Biochemical	10 (4.3 ± 1.3%)	1 (2.9 ± 2.8%)	2 (3.9 ± 2.8%)	3 (3.4 ± 1.8%)	4 (7.3 ± 3.7%)	
1 sac	143 (62.2 ± 3.3%)	21 (60.0 ± 8.7%)	29 (56.9 ± 7.1%)	59 (66.3 ± 4.8%)	34 (61.8 ± 6.7%)	
2 sacs	63 (27.4 ± 2.8%)	9 (25.7 ± 7.6%)	18 (35.3 ± 6.8%)	21 (23.6 ± 4.4%)	15 (27.3 ± 6.1%)	
3 sacs	14 (6.1 ± 1.6%)	4 (11.4 ± 5.5%)	2 (3.9 ± 2.7%)	6 (6.7 ± 2.7%)	2 (3.6 ± 2.6%)	

Results are presented as frequencies, percent and standard error, or means and standard deviation. Differences between groups were calculated using either the Chi-Square test for categorical data, an ANOVA with a post hoc Bonferroni test for parametric, continuous data, or the Kruskal-Wallis test with a *post hoc* Dunn's test for non-parametric, continuous data. A significant difference between the groups ($p < 0.05$) are indicated by an asterisk (*).

^a vs. Donor normozoospermia sperm, $p < 0.05$.

^b vs. Paternal normozoospermia sperm, $p < 0.05$.

^c vs. Paternal sperm with teratozoospermia, $p < 0.05$.

(Table 2). Moreover, the sperm source (donor versus paternal) and the etiology present in the sperm sample (normozoospermic versus teratozoospermic versus severe abnormalities) had no difference in the clinical pregnancy rate and embryo implantation.

4. Discussion

In the present study, we assessed the age-dependent effects on semen quality between partner and donor samples with respect to embryo implantation. Independent of the semen source, we found no difference in the implantation rate. Moreover, the semen source's age did not affect the aneuploidy rates if the sperm was diagnosed as normozoospermic. This was a clinical retrospective study, which can only show an association and give credence to future hypotheses for experimental research.

Aneuploid embryos are associated with implantation failure [20, 21], where the ovum is associated with a more significant influence on the aneuploidy rate [22]. Here, using donor ova, the expected aneuploidy rate is between 20 and 30% [23, 24], about 25% of the analyzed embryos presented with aneuploidies. This rate was independent of the sperm source (paternal versus donor) and etiology (Fig. 2). Similar studies by Dubey *et al.* and Mazzilli *et al.* confirmed this result as they also observed that sperm morphological alterations do not affect the embryo aneuploidy rate [24–26]. The proposed explanation suggests that using ICSI still delivers an uncompromised genome and sperm alterations are independent of the sperm chromosome composition. Nevertheless, Coban *et al.* [27] demonstrated that the severity of the sperm

defect, as quantified by Kruger's criteria, can significantly affect the aneuploidy rate, which is also confirmed by Magli *et al.* [28]. Here, we observed a prevalence of sperm morphological defects in the analyzed samples (teratozoospermia etiology); however, none of the etiologies correlated with the types of aneuploidies. Still, a sub-analysis was not performed because some of the groups had few samples and significant heterogeneity.

Numerous studies demonstrate the maternal effect age has on aneuploidy rates [4, 29]. Nevertheless, the age of the sperm source has also been shown to have an effect on increasing embryo aneuploidies as well as reducing IVF success [7–9]. The effect of the semen source's age is still under debate [30]. Here, we observed no correlation in semen donor samples nor with partner samples. Although the samples were stratified by etiology into normal and abnormal sperm, only the teratozoospermia group positively associated the partner's age with the aneuploidy rate; moreover, there was a weak correlation between paternal age and the presence of sperm abnormalities. This could be an artifact as the prevalence for specific sperm etiologies is increased with age, and the aneuploidy rate is associated with sperm etiology [27, 31–33]. Even though our study does not confirm the latter effect, there is a suggested aneuploidy rate difference between certain types of sperm etiologies [27, 28, 34]. Magli *et al.* [28] demonstrated that the non-obstructive azoospermia and oligoasthenoteratozoospermia groups presented with more chromosomally compromised embryos than the normozoospermia group using fluorescence *in-situ* hybridization and, as mention above, Coban *et*

al. [27] confirmed this effect. A review by Caseiro *et al.* [35] nicely explains the association between oligoasthenoteratozoospermia and embryo aneuploidies. Lastly, teratozoospermia was shown to be associated with higher rates for trisomies and monosomies [34]. Still, here, the sample size was not sufficient to demonstrate this effect.

The contribution of spermatozoa to embryos aneuploidies in IVF has been the topic of several debates. Garcia-Ferreira *et al.* [9] showed that the aneuploidy rates from ova donation cycles determined a high incidence of aneuploidies when men are older than 50 years. Here, no significant difference was found between the ages of the males (paternal or donors) concerning aneuploidy rates in the analyzed embryos.

Several studies have reported the correlation between the aneuploidy rates in embryos derived from abnormal sperm. Coates *et al.* [36] determined that the aneuploidy incidence was significantly higher in embryos obtained from sperm with oligozoospermia etiology than those in embryos obtained from sperm with normal male factor, independent of the ova source. However, Mazzilli *et al.* [26] compared five different male etiologies (normozoospermic patients, patients with moderate male factor, patients with oligoasthenoteratozoospermia/crytozoospermia, patients with obstructive azoospermia, and patients affected with non-obstructive or secretory azoospermia) for their effects on fertilization, embryonic development, aneuploidies incidence, and gestational results. They found that early embryonic development was impaired in patients with severe male factors with respect to fertilization rates and development potential; however, the euploidy rate and blastocyst implantation potential were independent of the sperm quality. They concluded that the male factor would not be an indication when suggesting Preimplantation Genetic Testing (PGT) for an IVF cycle. In the present study, no significant differences were found regarding embryonic development results (fertilization and blastocyst formation rate) as well as with pregnancy and implantation rates, when compared to patients with normal or altered male factor. Our result is in agreement with Mazzilli *et al.* [26].

Due to the oocyte's contribution to the aneuploidy rate, under certain circumstances, such as advanced maternal age, embryos are analyzed by PGT-A. In the present study, we focused on evaluating the impact of the male factor in terms of age and seminal quality in exclusively ova donation cycles. Although no significant correlation was found between age and aneuploidy incidence nor between seminal quality and aneuploidies incidence, we did observe an effect of a male factor on aneuploidy rates. Therefore, it would be beneficial for these patients to consider PGT-A.

This study has a few limitations. First, we did not quantify or qualify the type of embryo aneuploidies. Only euploid embryos were transferred. However, specific aneuploidies could be generated and, through the embryo's auto-correcting mechanisms, produce a viable euploid fetus [37, 38]. Second, the severity of the sperm deformities was not

quantified in samples with teratozoospermia diagnosis or other severe alterations. As mentioned above, the effects of sperm with abnormal etiology are associated with higher aneuploidy rates. Third, only the donor semen samples were frozen until use, whereas patient samples were fresh. The effect of cryopreservation on sperm's DNA integrity is still unclear and under debate [39]. Fourth, the reasons for oocyte donation were not considered, such as recurrent implantation failure, low reserve, or inability to produce viable embryos, to name a few. Underlining the mechanism associated with each pathology could introduce bias to the results. Fifth, all embryos underwent PGT-A using NGT; therefore, these results are limited to this group. Lastly, this is a clinical retrospective study in which we can show an association but not a causal relationship.

5. Conclusions

Here, we examine if the sperm source's age affects the aneuploidy rate determined by NGS-PGT-A, which it does not; however, with the teratozoospermia diagnosis, there was a weak association. Therefore, it would be prudent to perform PGT-A when advanced age and teratozoospermia etiology are concurrent. Lastly, low-quality semen did not affect embryo implantation. Overall, these results promote the use of PGT-A.

Abbreviations

BMI, Body-mass index; FSH, Follicle-stimulating hormone; GnRH, Gonadotropin-releasing hormone; hCG, Human chorionic gonadotropin; HSA, Human serum albumin; HTF, human tubal fluid; ICSI, intracytoplasmic sperm injection; IVF, *in vitro* fertilization; NGS, Next-Generation Sequencing; PCR, Polymerase chain reaction; PGT-A, Preimplantation Genetic Testing for Aneuploidies; WGA, Whole-genome amplification.

Author contributions

JP and ELB conceived and designed the study; JP, HS, and JC performed the experiments, data collection as well as monitored the patients; JP and ELB analyzed the data; JP and ELB wrote the manuscript and critically analyzed it; ELB supervised the study and was responsible for the funding. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

In this retrospective study, no written informed consent was needed besides the general consent for data utilization; nevertheless, the study was conducted following the Declaration of Helsinki. The Ethics Committee of the Institute Ingenes México approved this study (approval number: ISF120516).

Acknowledgment

We want to express our gratitude to the study participants and the medical staff at Ingenes Institute. We thank Dr. Leonardo M. Porchia for drafting the manuscript and Ing. Lucero Cervantes for her editorial assistance.

Funding

The study was funded by Consejo Nacional de Ciencia y Tecnología (Conacyt grant number: 250768) to ELB. The funding source did not have a role in the study design, collection, analysis, and interpretation of data and no part in the report's writing and submitting the paper for publication.

Conflict of interest

The authors declare no conflict of interest.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at <https://ceog.imrpess.com/EN/10.31083/j.ceog4806222>.

References

- [1] Kumar N, Singh AK. Trends of male factor infertility, an important cause of infertility: a review of literature. *Journal of Human Reproductive Sciences*. 2015; 8: 191–196.
- [2] Sharlip ID, Jarow JP, Belker AM, Lipshultz LI, Sigman M, Thomas AJ, *et al*. Best practice policies for male infertility. *Fertility and Sterility*. 2002; 77: 873–882.
- [3] Kidd SA, Eskenazi B, Wyrobek AJ. Effects of male age on semen quality and fertility: a review of the literature. *Fertility and Sterility*. 2001; 75: 237–248.
- [4] Cimadomo D, Fabozzi G, Vaiarelli A, Ubaldi N, Ubaldi FM, Rienzi L. Impact of Maternal Age on Oocyte and Embryo Competence. *Frontiers in Endocrinology*. 2018; 9: 327.
- [5] Ubaldi FM, Cimadomo D, Vaiarelli A, Fabozzi G, Venturella R, Maggiulli R, *et al*. Advanced maternal age in IVF: still a challenge? The present and the future of its treatment. *Frontiers in Endocrinology*. 2019; 10: 94.
- [6] Grøndahl ML, Christiansen SL, Kesmodel US, Agerholm IE, Lemmen JG, Lundstrøm P, *et al*. Effect of women's age on embryo morphology, cleavage rate and competence—a multicenter cohort study. *PLoS ONE*. 2017; 12: e0172456.
- [7] Colaco S, Sakkas D. Paternal factors contributing to embryo quality. *Journal of Assisted Reproduction and Genetics*. 2018; 35: 1953–1968.
- [8] Dain L, Auslander R, Dirnfeld M. The effect of paternal age on assisted reproduction outcome. *Fertility and Sterility*. 2011; 95: 1–8.
- [9] García-Ferreira J, Luna D, Villegas L, Romero R, Zavala P, Hilaro R, *et al*. High Aneuploidy Rates Observed in Embryos Derived from Donated Oocytes are Related to Male Aging and High Percentages of Sperm DNA Fragmentation. *Clinical Medicine Insights. Reproductive Health*. 2015; 9: 21–27.
- [10] Salmon-Divon M, Shrem G, Balayla J, Nehushtan T, Volodarsky-Perel A, Steiner N, *et al*. An age-based sperm nomogram: the McGill reference guide. *Human Reproduction*. 2020; 35: 2213–2225.
- [11] Harris ID, Fronczak C, Roth L, Meacham RB. Fertility and the aging male. *Reviews in Urology*. 2011; 13: e184–e190.
- [12] Petersen CG, Mauri AL, Vagnini LD, Renzi A, Petersen B, Mattila M, *et al*. The effects of male age on sperm DNA damage: an evaluation of 2,178 semen samples. *JBRA assisted reproduction*. 2018; 22: 323.
- [13] Oliveira JBA, Petersen CG, Mauri AL, Vagnini LD, Baruffi RLR, Franco Jr. JG. The effects of age on sperm quality: an evaluation of 1,500 semen samples. *JBRA Assisted Reproduction*. 2014; 18: 34–41.
- [14] Pino V, Sanz A, Valdés N, Crosby J, Mackenna A. The effects of aging on semen parameters and sperm DNA fragmentation. *JBRA Assisted Reproduction*. 2020; 24: 82.
- [15] Kamath MS, Antonisamy B, Selliah HY, La Marca A, Sunkara SK. Perinatal outcomes following IVF with use of donor versus partner sperm. *Reproductive Biomedicine Online*. 2018; 36: 705–710.
- [16] World Health Organization W. Laboratory Manual for the Examination and Processing of Human Semen. World Health Organization. 2010.
- [17] Kruger TF, Ackerman SB, Simmons KF, Swanson RJ, Brugo SS, Acosta AA. A quick, reliable staining technique for human sperm morphology. *Archives of Andrology*. 1987; 18: 275–277.
- [18] Gardner DK, Schoolcraft WB. Culture and transfer of human blastocysts. *Current Opinion in Obstetrics & Gynecology*. 1999; 11: 307–311.
- [19] Kuwayama M. Highly efficient vitrification for cryopreservation of human oocytes and embryos: the Cryotop method. *Theriogenology*. 2007; 67: 73–80.
- [20] Emery BR, Wilcox AL, Aoki VW, Peterson CM, Carrell DT. *In vitro* oocyte maturation and subsequent delayed fertilization is associated with increased embryo aneuploidy. *Fertility and Sterility*. 2005; 84: 1027–1029.
- [21] Hoyos LR, Cheng CY, Brennan K, Hubert G, Wang B, Buyalos RP, *et al*. Euploid rates among oocyte donors: is there an optimal age for donation? *Journal of Assisted Reproduction and Genetics*. 2020; 37: 589–594.
- [22] Sills ES, Li X, Frederick JL, Khoury CD, Potter DA. Determining parental origin of embryo aneuploidy: analysis of genetic error observed in 305 embryos derived from anonymous donor oocyte IVF cycles. *Molecular Cytogenetics*. 2014; 7: 68.
- [23] Rosenwaks Z, Handyside AH, Fiorentino F, Gleicher N, Paulson RJ, Schattman GL, *et al*. The pros and cons of preimplantation genetic testing for aneuploidy: clinical and laboratory perspectives. *Fertility and Sterility*. 2018; 110: 353–361.
- [24] Schaeffer E, Porchia LM, Neumann A, Luna A, Rojas T, López-Bayghen E. Embryos derived from donor or patient oocytes are not different for *in vitro* fertilization outcomes when PGT allows euploid embryo selection: a retrospective study. *Clinical and Translational Medicine*. 2020; 9: 14.
- [25] Dubey A, Dayal MB, Frankfurter D, Balazs P, Peak D, Gindoff PR. The influence of sperm morphology on preimplantation genetic diagnosis cycles outcome. *Fertility and Sterility*. 2008; 89: 1665–1669.
- [26] Mazzilli R, Cimadomo D, Vaiarelli A, Capalbo A, Doveire L, Alviggi E, *et al*. Effect of the male factor on the clinical outcome of intracytoplasmic sperm injection combined with preimplantation aneuploidy testing: observational longitudinal cohort study of 1,219 consecutive cycles. *Fertility and Sterility*. 2017; 108: 961–972.e3.
- [27] Coban O, Serdarogullari M, Onar Sekerci Z, Bilgin EM, Serakinci N. Evaluation of the impact of sperm morphology on embryo aneuploidy rates in a donor oocyte program. *Systems Biology in Reproductive Medicine*. 2018; 64: 169–173.
- [28] Magli M, Gianaroli L, Ferraretti A, Gordts S, Fredericks V, Crippa A. Paternal contribution to aneuploidy in preimplantation embryos. *Reproductive BioMedicine Online*. 2009; 18: 536–542.
- [29] Demko ZP, Simon AL, McCoy RC, Petrov DA, Rabinowitz M. Effects of maternal age on euploidy rates in a large cohort of embryos analyzed with 24-chromosome single-nucleotide polymorphism-based preimplantation genetic screening. *Fertility and Sterility*. 2016; 105: 1307–1313.
- [30] Rolland M, Le Moal J, Wagner V, Royère D, De Mouzon J. Decline in semen concentration and morphology in a sample of 26,609 men close to general population between 1989 and 2005 in France. *Human Reproduction*. 2013; 28: 462–470.

- [31] Brahem S, Mehdi M, Elghezal H, Saad A. The effects of male aging on semen quality, sperm DNA fragmentation and chromosomal abnormalities in an infertile population. *Journal of Assisted Reproduction and Genetics*. 2011; 28: 425–432.
- [32] Silva LFI, Oliveira JBA, Petersen CG, Mauri AL, Massaro FC, Cavagna M, *et al*. The effects of male age on sperm analysis by motile sperm organelle morphology examination (MSOME) *Reproductive Biology and Endocrinology*. 2012; 10: 19.
- [33] Sun F, Ko E, Martin RH. Is there a relationship between sperm chromosome abnormalities and sperm morphology? *Reproductive Biology and Endocrinology*. 2006; 4: 1.
- [34] Kiseleva Y, Abubakirov A. Teratozoospermia is associated with the embryo aneuploidy and leads to the sex ratio imbalance. *Fertility and Sterility*. 2017; 108: e127.
- [35] Caseiro AL, Regalo A, Pereira E, Esteves T, Fernandes F, Carvalho J. Implication of sperm chromosomal abnormalities in recurrent abortion and multiple implantation failure. *Reproductive BioMedicine Online*. 2015; 31: 481–485.
- [36] Coates A, Hesla JS, Hurliman A, Coate B, Holmes E, Matthews R, *et al*. Use of suboptimal sperm increases the risk of aneuploidy of the sex chromosomes in preimplantation blastocyst embryos. *Fertility and Sterility*. 2015; 104: 866–872.
- [37] Barbash-Hazan S, Frumkin T, Malcov M, Yaron Y, Cohen T, Azem F, *et al*. Preimplantation aneuploid embryos undergo self-correction in correlation with their developmental potential. *Fertility and Sterility*. 2009; 92: 890–896.
- [38] Bazrgar M, Gourabi H, Valojerdi MR, Yazdi PE, Baharvand H. Self-correction of chromosomal abnormalities in human preimplantation embryos and embryonic stem cells. *Stem Cells and Development*. 2013; 22: 2449–2456.
- [39] Kopeika J, Thornhill A, Khalaf Y. The effect of cryopreservation on the genome of gametes and embryos: principles of cryobiology and critical appraisal of the evidence. *Human Reproduction Update*. 2015; 21: 209–227.