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MALE REPRODUCTION: ORIGINAL ARTICLE

Male Age and Progressive Sperm Motility Are Critical Factors Affecting Embryological and Clinical Outcomes in Oocyte Donor ICSI Cycles

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Abstract

This retrospective cohort study aimed to explore whether paternal age and semen quality parameters affect the embryological and clinical outcomes of ICSI with oocyte donation. A total of 339 oocyte donation (OD)-ICSI cycles were categorized into four groups according to the semen parameter profiles of the male counterparts: normozoospermia (NS, $n=184$), oligozoospermia (OS, $n=41$), asthenozoospermia (AS, $n=50$), and oligoasthenozoospermia (OAS, $n=64$). The effect of age, total sperm count, and progressive motility was separately analyzed for reproductive outcomes and compared between the study groups: fertilization, blastulation, and top-quality embryo rate, biochemical and clinical pregnancy, live birth, and miscarriage. A negative correlation between male age and fertilization rate was observed ($r_s=-0.23$, $p<0.0001$), while male age was a significant factor for biochemical pregnancy ($p=0.0002$), clinical pregnancy ($p=0.0017$), and live birth ($p=0.0038$). Reduced total sperm count and lowered progressive motility led to poorer fertilization rates ($r_s=0.19$ and 0.35, respectively, $p<0.0001$) and affected embryo quality ($r_s=0.13$, $p=0.02$, and $r_s=0.22$, $p<0.0001$, respectively). OD-ICSI cycles with asthenozoospermia had significantly lowered success rates in biochemical pregnancy, clinical pregnancy, and live birth ($p<0.05$). Our study demonstrated that both advanced male age and reduced progressive motility of spermatozoa exert a significant negative influence on the outcome of assisted reproduction, even in controlled procedures with gamete selection and optimization such as in OD-ICSI. Improvement in treatment strategies and male fertility evaluation requires incorporation of such evidence to obtain better prognosis towards personalized management.

Keywords Male infertility · Paternal age · Semen analysis · Oocyte donation · IVF · ICSI

Introduction

The global health problem of infertility affects 8–12% of couples in reproductive age [1, 2], with male factor being involved in more than half of the cases, corresponding to about 30 million men worldwide and an overall estimation of 2.5 to 12% of the total male population [3]. The introduction of intracytoplasmic sperm injection (ICSI) [4] and its variants has allowed a positive reproductive outcome even in cases where low semen quality would severely affect

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the chances of successful reproduction through spontaneous conception or less invasive assisted reproductive technologies (ART). Theoretically, the retrieval, isolation, and manipulation of a single "functional" spermatozoon under high magnification should circumvent male factor and lead to the creation of a viable embryo progressing to live birth; however, a summary of European registries reports that clinical pregnancy still highs at a rate of 35% in experienced clinics including patients with good prognosis [5].

A spermatozoon is more than a vessel to provide a haploid genome [6], as it maintains a central role in crucial embryological procedures following fertilization, by regulating embryogenesis, developmental progression, and epigenetics [7]. This also may be depicted through reports claiming a poor clinical outcome in 30% of men presenting with infertility [8] that exhibit a normal semen analysis based on the World Health Organization (WHO) 2010 criteria [9]. However, the investigation of the paternal factor has been partly "neglected" due to the possibility of performing in vitro treatments, the relative abundance of male gametes over female, the advancement of sperm retrieval techniques, and the notably good cryobiological behavior of sperm during long-term storage. All these aspects have limited our understanding on the male confounding factors that might preclude ART success.

Several aspects in terms of diagnosis and etiology of male infertility and paternal effects in reproduction remain obscure [10], and fertility evaluation relies mainly on a set of baseline characteristics defined by the WHO, with sperm concentration, motility, and normal morphology as a probabilistic assessment of reproductive potential [9]. In the pursuit of defining the "successful" spermatozoon, additional factors and assays have emerged examining the genetic integrity and function of the sperm, to standardize an innovative evaluation that would provide cohesive information on its status. Biomarkers with variable sensitivity have been utilized over the course of ART evolution, such as DNA fragmentation and other function tests that provide valuable information on gamete interaction and reproductive potency [11, 12], while a future importance has been outlined for genomics, epigenetics, metabolomics, and proteomics [13].

By investigating ICSI treatment success, conflicting views emerge as to whether male age and semen quality correlate with embryological and clinical outcomes. Male age has been investigated in multiple instances, and on one side, data shows an association between advanced male age with declined sperm quality and poor clinical outcomes [6, 14–17], while on the other side, other studies report no association of age with clinical parameters [18, 19] or sperm parameters [20–22]. In terms of semen quality, several studies have reported no association of sperm parameters (i.e., count, motility, and morphology) with embryological and clinical outcomes following ICSI [19, 23–27], while others indicate a weak association with some outcomes [18, 22, 28, 29]. On the contrary, several studies support a strong negative influence of abnormal sperm characteristics in clinical outcomes following ART [6, 17, 20, 21, 30, 31]. The evident inconsistency in the findings of previous reports may affect fertility evaluation and management and can be attributed to the noted variabilities in the study design, population characteristics, infertility factors, sperm sources, fertilization methods, ART cycle parameters, study outcomes, semen quality assessment methods and cut-off values, inclusion and exclusion criteria, and methods of analysis.

These controversies corroborate with the notion that we should attempt to gain a better insight into the characteristics of the "successful" sperm and to establish prognosis in the anticipated outcomes of ART. This should allow optimization of embryological methods and clinical results and furthermore encourage a patient-centered management with better prognostic criteria for adequate consultation of the infertile individuals and couples. The rapid technological evolution alongside the vast availability of data deriving from IVF/ICSI cycles tends to skew our scientific interests in novel factors for deciphering the complexity of male factor and its contribution in reproductive outcomes. It is thus challenging to return to simplistic research questions and support that basic sperm parameters may possess a more complex counterpart in procedures that we so far thought that circumvented male factor such as ART and specifically ICSI. Therefore, the principal aim of this study is to investigate the relationship between different sperm parameters and reproductive outcomes of ART, following ICSI with donor oocytes. Specifically, we compare the reproductive outcomes of normozoospermic, oligozoospermic, asthenozoospermic, and oligoasthenozoospermic patients, as defined by the WHO 2010 criteria, from OD-ICSI cycles. In addition, as male age has been implied to influence these parameters, we analyze its possible associations with fertility status and clinical outcomes for improved infertility management.

Population and Methods

Ethical Approval

The study was approved by the Research and Ethics Committee of the Reproductive Center (EVD 0701/2020) and has been conducted in accordance with the ethical standards of the National Authority for Medically Assisted Reproduction and with the 1964 Helsinki Declaration and its later amendments [32].

Study Design, Population, and Participant Characteristics

Data was retrospectively collected from 243 cases diagnosed with infertility, undergoing a total of 339 OD-ICSI cycles, including 37 cycles with donor sperm. The study included cycles conducted from January 5th, 2015, to August 30th, 2019, at the fertility clinic. Patients were referred to the clinic for isolated male/female or combined factors of infertility, unexplained infertility, or repeated implantation failure following IVF/ICSI. Patients received extensive consultation throughout the stages of treatment and written consent was obtained by all participants.

Anonymous non-smoking oocyte donors ($n = 285$) between 21 and 30 years of age volunteered altruistically for a third party ICSI treatment as aligned with the National Regulations [33] and were screened according to the routine procedures for follicle stimulating hormone (FSH < 9 mIU/mL), luteinizing hormone (LH < 12 mIU/mL), estradiol (E2 < 9 0 pg/mL), antral follicle count (AFC > 10), and body mass index (BMI < 29 kg/m^2).

Recipients were females ($n = 243$) between 29 and 49 years of age attending the clinic for treatment with OD, with no endometrial or endocrinological pathologies and without any medical history of endometriosis, hydrosalpinx, or autoimmune disorders. Sperm samples were collected from male partners, in addition to 37 cases where donor sperm was used, with the age span of the male partners/sperm donors lying between 21 and 59 years. From the total number of cycles, 285 underwent one embryo transfer (ET), while 54 cycles had subsequent ETs from previously cryopreserved surplus embryos. Both partners and the participants in the donation program were free from genetic abnormalities and prevalent conditions to the local population, such as thalassemia and tested negative for sexually transmitted diseases (human immunodeficiency virus, hepatitis, syphilis).

Criteria for exclusion from analysis were surrogacy, embryo biopsy for preimplantation genetic testing (PGT) for aneuploidies, structural rearrangements and monogenic defects, cases with incomplete data that failed to follow-up, semen analysis characteristics with complete azoospermia or 0% morphologically normal sperm in the ejaculate according to strict criteria [34], and cycles with sperm retrieval through other sources than voluntary ejaculation (electroejaculation, surgical sperm aspiration, or extraction). The fertility treatment protocols of the clinic comply with the notion that any urogenital disorder is addressed prior to cycle initiation; therefore, male participants did not present with any genitourinary infections or other reproductive pathologies that have not been attended and could influence clinical outcomes. The male population of study participants did not include any subjects with medical history of malignancies or previous chemotherapy and/or radiotherapy. In addition, cycles that received adjuvant treatments that could affect the outcome were excluded.

Medical and Embryological Procedures

The OD program involved controlled ovarian hyperstimulation (COH) with a standard antagonist protocol. An average of 2,000 IU/mL recombinant FSH (r-FSH) (Puregon, Merck Sharp & Dohme, Haarlem, Netherlands) was administered per cycle, combined with gonadotropin-releasing hormone (GnRH) antagonist (Orgalutran by Merck Sharp & Dohme, Haarlem, Netherlands) for the final maturation of stimulated follicles. Triggering of ovulation was performed 34–36 h before ultrasound-guided oocyte retrieval, with 250 µg choriogonadotropin alpha (Ovitrelle, Merck Serono Europe Limited, London, UK). Donor oocyte recipients were prepared for endometrial transfer of the embryo/s, through progesterone administration with a combination of oral capsules (Utrogestan, Faran Laboratories AVEE, Attica, Greece) and intramuscular injection (Prolutex, IBSA Farmaceutici Italia, Lodi, Italy). ET was performed under continuous ultrasound guidance for adequate embryo deposition with Wallace catheters (CooperSurgical, Malov, Denmark).

For ICSI procedures, sperm samples were collected through masturbation after adequate ejaculatory abstinence (2–7 days) and assessed following liquefaction, according to the WHO guidelines for the examination and processing of human semen [9]. Semen samples were prepared by means of density gradient centrifugation at room temperature for 20 min at 300 × *g* (Sydney IVF Sperm Gradient, COOK Medical, Bloomington, IN, USA), and spermatozoa-containing pellets were re-suspended in appropriate gamete handling medium (Origio Universal IVF Medium, CooperSurgical, Malov, Denmark). Processed samples were cryopreserved with the addition of one part of cryoprotectant with 5% glycerol (Sperm CryoProtec, Nidacon International AB, Molndal, Sweden) to three parts of sperm sample and slow freezing in liquid nitrogen vapor. Thawing was performed on the day of oocyte retrieval of the donor, as instructed by cryoprotectant manufacturer protocol with the direct suspension of the container straw in water at 37 °C for 30 s, with recovery rate of > 50% of the total motility, as reported by the manufacturer (Nidacon International AB, Molndal, Sweden).

Sperm quality parameters (concentration, total sperm count, and progressive motility) were assessed according to WHO guidelines [9]. Morphology was not evaluated because the standard embryological procedures of the center preclude the use of dye to assess strict morphology, on specimens destined for ICSI. Uniformity of the treatments and procedures was assured by morphological sperm selection under high magnification during the ICSI procedure

for insemination of donor oocytes, with all included cycles being performed with semen samples of > 0% morphologically normal sperm.

A maximum of two embryos per transfer was optioned and allowed by the National Legislation [33], with the embryos being transferred at blastocyst stage and scored according to the Gardner system [35]. Embryos were ranked according to their morphology as "top-quality" by visualizing a tightly packed inner cell mass with many cells and an outer cell mass composed by many cells, forming a cohesive epithelium. In cases with subsequent transfers, best quality embryos were transferred first.

Study Variables

For the analysis of the results, the included OD-ICSI cycles were categorized based on the sperm parameters in the following groups: oligozoospermia (total sperm count $< 39 \times 10^6$ sperm), asthenozoospermia (progressive motility < 32%), oligoasthenozoospermia (total sperm count $< 39 \times 10^6$ sperm, progressive motility < 32%), and normozoospermia (total count $\geq 39 \times 10^6$ sperm, progressive motility ≥ 32%), according to the WHO criteria [9].

Clinical Outcomes

Clinical outcomes were defined according to the International Glossary on Infertility and Fertility Care [36]. Biochemical pregnancy was defined as a pregnancy confirmed by the detection of beta human chorionic gonadotropin (β-hCG) in serum, 12–14 days following ET. Clinical pregnancy was diagnosed by the visualization of one or more gestational sacs through ultrasound with the presence of fetal heartbeat. Live birth was accounted as the complete expulsion or extraction of an individual or twins, after 22 completed weeks of gestation with definitive signs of life, whereas miscarriage was defined as the spontaneous loss of a clinical pregnancy before 22 weeks of gestation with non-viable fetus/es.

A negative outcome was considered in cases where there was no notable rise in serum β-hCG following ET, while cases with borderline values were confirmed by re-evaluation using fresh samples 48 h later. All clinical outcomes were examined per transfer. In addition, embryological outcomes were available for each semen parameter group, and thus, fertilization, blastulation, and top embryo quality rates were also included in the primary analysis. All embryological outcomes were analyzed per cycle.

Data Collection and Statistical Analysis

Eligible ICSI cycle data, from the oocyte donation program of the center, were recorded in an Excel spreadsheet format and imported to Microsoft Excel 2010. Anonymity of the data and privacy protection was ensured by the assignment of an exclusive code for each participant and matching it with the corresponding ICSI cycle/s and the respective outcomes. Access to the data and the assignment process was performed by authorized scientific personnel participating in the study by signing a confidentiality agreement.

Statistical analysis was performed by using SAS for Windows 9.4 software platform (SAS Institute Inc., Campus Drive Cary, NC, USA). Depending on the normality of distribution, numerical data was either expressed as the median value with the interquartile range (IQR) or as a mean ± standard deviation (SD), with minimum and maximum values. For categorical data, the relevant percentages within individual groups were reported. Non-parametric tests were applied, specifically the Kruskal–Wallis and Mann–Whitney *U* test, while comparison of categorical variables was performed through the χ^2 test and Fisher exact test. Bivariate categorical data was assessed through odds ratio (OR) with the corresponding 95% confidence interval (CI). In order to adjust for the female recipient age, we applied logistic regression models for the prediction of the four main outcomes of the study stratified by sperm parameters and female age; specifically, we applied forward selection models with a threshold of $p < 0.05$. The reference level for the sperm parameter status was normozoospermia. Correlation was evaluated by the Spearman (r_s) correlation coefficient. For multiple comparisons, Bonferroni correction was applied. A p value of < 0.05 was considered as statistically significant.

Results

Demographic and Cycle Characteristics

The mean age of oocyte donors was 24.4 ± 2.0 years, and a mean BMI of 22.2 ± 1.6 kg/m^2 was determined by measurements at the occurrence of procedures. OD recipients had a mean age of 43.5 ± 4.0 years, with 58.4% of the cohort having no previous pregnancies, and 96.2% had no reproductive history with either birth or stillbirth. For the majority of the participating women in this retrospective analysis, this was their first OD-ICSI attempt in the reporting center ($n = 243$, 71.7%). Sperm samples were acquired from males with a mean age of 42.5 ± 7.2 years. Full demographic and cycle characteristics of the study population are presented in Table 1.

All transfers to the endometrial cavity were performed at blastocyst stage, on day 5 of embryonic development, and no statistical difference was found between study groups in the number of transferred embryos (mean 1.9 ± 0.3), with 90% of the cycles undergoing a double ET. The mean

Table 1 Demographic characteristics of participants, with descriptive data on oocyte donation (OD)-ICSI cycles and embryological characteristics. SD, standard deviation; Q, quartile; MII, metaphase II; 2PN, presence of two pronuclei; D5, day 5 of embryonic development

Group	Variable	Mean	SD	Min	Max	Median	Q1	Q3
Demographics	Age, female partner/recipient	43.50	3.96	29	49	44	41	46
	Age, male partner	42.50	7.15	21	59	44	41	47
	Age, oocyte donor	24.35	2.02	21	30	24	23	26
	BMI, oocyte donor	22.22	1.57	18.5	24.9	22.3	20.9	23.5
	BMI, male partner	25.99	2.13	21.2	32.0	26.1	24.2	27.4
	Gravidity, female partner/recipient	0.54	0.73	0	3	0	0	1
	Parity, female partner/recipient	0.04	0.20	0	1	0	0	0
OD cycle	Allocated oocytes per cycle	9.91	2.53	5	22	10	8	11
	Number of mature (MII) oocytes	7.57	2.12	3	18	8	6	8
	Number of fertilized oocytes (2PN)	5.77	1.85	2	14	6	5	7
	Number of ETs	1.37	0.65	1	4	1	1	2
	Consecutive ETs (vitrified embryos, D5)	0.17	0.40	0	2	0	0	0
Embryological data	Number of blastocysts	3.41	1.29	1	7	4	2	4
	Top-quality embryos (D5)	1.56	0.99	0	4	2	1	2
	Number of embryos transferred (D5)	1.90	0.30	1	2	2	2	2
	Surplus embryos vitrified per cycle (D5)	1.07	1.22	0	5	1	0	2

number of conducted ETs per patient was 1.4 ± 0.7, and a mean 1.1 ± 1.2 of surplus embryos was competent for vitrification and transfer in consecutive cycles. From the present cohort of OD-ICSI cycles, only a small fraction (14%) had no embryos with top morphological quality available for transfer. In contrast, 86% of the cycles had at least one embryo competent for implantation with top-quality morphological characteristics.

During the initial evaluation of semen quality, a total of 54.3% samples were normozoospermic ($n = 184$), while oligozoospermia was observed in 12.1% ($n = 41$), asthenozoospermia in 14.8% ($n = 50$), and oligoasthenozoospermia in 18.9% of the cases ($n = 64$). Semen parameters of concentration, total sperm count, progressive, and total motility for each allocated group in the study are summarized in Table 2.

Paternal Age

The median age (Q1-Q3) of normozoospermic, oligoasthenozoospermic, oligozoospermic, and asthenozoospermic patients was 42 (38–46), 45 (43–47.5), 44 (41–46),

Table 2 Sperm parameters in each group of patients according to initial assessment on fresh samples. NS, normozoospermia; OS, oligozoospermia; AS, asthenozoospermia; OAS, oligoasthenozoospermia; SD, standard deviation; Q, quartile

Sperm status	Sperm characteristic	Mean	SD	Min	Max	Median	Q1	Q3
NS	Concentration ($\times 10^6$/ml)	49.61	25.67	15.00	160.00	45.00	32.00	61.00
	Total sperm count ($\times 10^6$)	124.30	87.26	40.60	560.00	102.10	71.40	145.50
	Progressive motility (%)	42.03	5.55	32.00	57.00	41.00	38.00	46.00
	Total motility	61.20	7.49	40.00	91.00	61.00	57.00	67.00
OS	Concentration ($\times 10^6$/ml)	16.44	8.97	2.00	35.00	14.00	10.00	20.00
	Total sperm count (10^6)	23.69	10.31	4.20	38.00	22.40	16.00	36.00
	Progressive motility (%)	40.41	6.07	32.00	58.00	38.00	36.00	45.00
	Total motility	59.07	6.96	46.00	72.00	58.00	54.00	65.00
AS	Concentration ($\times 10^6$/ml)	42.88	28.28	10.00	108.00	29.00	20.00	64.00
	Total sperm count ($\times 10^6$)	101.06	87.87	39.00	468.00	65.60	44.00	112.00
	Progressive motility (%)	19.66	7.02	6.00	31.00	21.00	13.00	25.00
	Total motility	33.44	7.90	11.00	46.00	34.00	27.00	40.00
OAS	Concentration ($\times 10^6$/ml)	7.29	7.38	0.10	36.00	6.00	0.90	11.00
	Total sperm count ($\times 10^6$)	14.20	12.30	0.18	37.00	13.30	1.45	24.00
	Progressive motility (%)	16.70	8.68	0.00	30.00	17.00	10.00	24.00
	Total motility	29.88	10.32	5.00	44.00	34.50	21.00	37.00

and 47 (44–48) years, respectively. No significant difference was observed between asthenozoospermia and oligoasthenozoospermia ($p = 0.2732$), although these groups were statistically different compared to the median age of normozoospermia ($p < 0.0001$) and asthenozoospermia group was different to oligozoospermia ($p = 0.0028$) (Supp. Fig. 1). Spearman analysis revealed a significant negative correlation of male age and progressive motility ($r_s = -0.30$, $p < 0.0001$), as well as total motility ($r_s = -0.29$, $p < 0.0001$), and sperm concentration ($r_s = -0.12$, $p = 0.0251$), while no association could be determined between age and total sperm count ($r_s = -0.12$, $p = 0.0921$).

In terms of clinical and embryological outcomes, paternal age significantly affected fertilization ($r_s = -0.23$, $p < 0.0001$), whereas this result could not be reproduced for embryological metrics of blastulation ($r_s = -0.09$, $p = 0.2814$) and top embryo quality rates ($r_s = -0.06$, $p = 0.8208$) that appeared unaffected by the age of the male partners/sperm donors. Advanced paternal age was directly associated with clinical outcomes through Mann–Whitney *U* test, as median age was found to be significantly increased separately for biochemical pregnancy ($p = 0.008$), clinical pregnancy ($p = 0.0068$), and live birth ($p = 0.0152$) in cycles that produced negative clinical results. For the incidence of miscarriage, no difference in male age could justify an association ($p = 1.000$) (Fig. 1, Supp. Table 1).

Semen Quality Parameters

To investigate the impact of sperm parameters on the reported embryological outcomes, statistical comparisons were performed with Mann–Whitney *U* test between the stratified groups for fertilization, blastulation, and top-quality embryo rates (Fig. 2, Supp. Table 2). The analysis revealed that total sperm count and progressive and total motility had a significant impact on fertilization rate and on the percentage of the formation of top-quality embryos ($p < 0.0001$), although without a visible effect on blastulation rate ($p > 0.05$). Asthenozoospermic samples exhibited the lowest fertilization rate (61.29%) and compromised embryo quality with only 30.4% of blastocysts being morphologically competent, having additionally the widest quartile range (0–50%) on embryo quality (Fig. 2).

Following ET, a positive serum β-hCG was measured in 242 cycles (71.4%), as an early implantation precursor, and clinical pregnancy was confirmed in 218 cycles (64.3%), of which 175 (51.6%) pregnancies progressed to live birth, with no adverse perinatal outcomes for the entire cohort. In total, 120 singletons and 55 twins were born. A categorical comparison of normal against abnormal sperm parameters revealed that the cycles with normozoospermic samples had 1.5 times higher odds to result in a positive clinical outcome; however, an independent break down on the clinical rates would amply support a borderline significance for clinical pregnancy ($p = 0.048$), a trend for biochemical pregnancy ($p = 0.065$), and no difference in live birth ($p = 0.126$) (Supp.

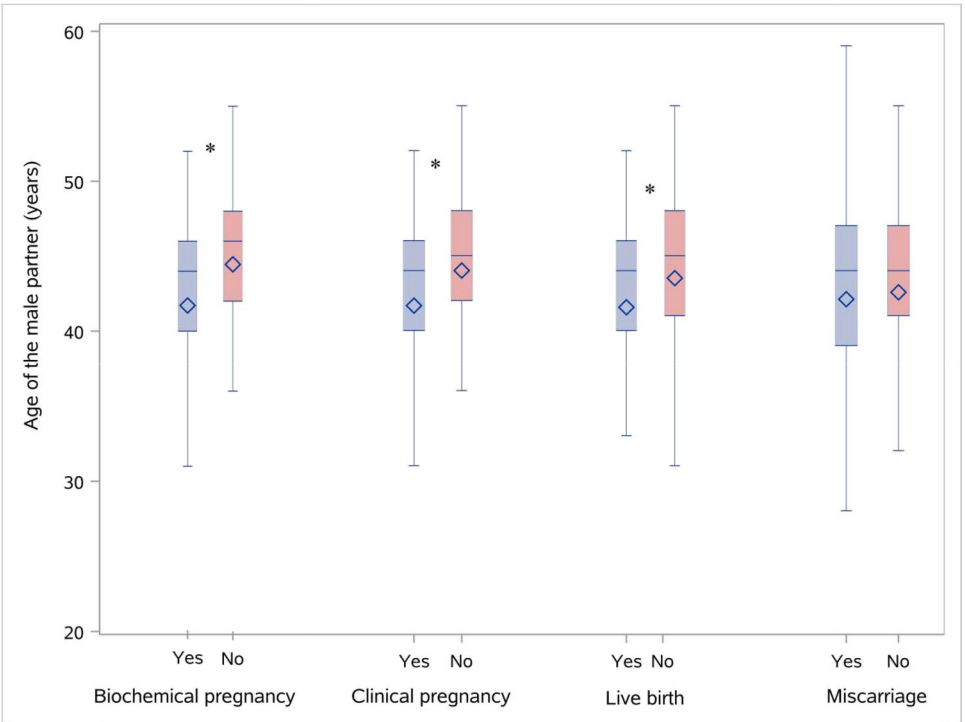

Fig. 1 Box and whisker plots comparing the paternal age in the individual clinical outcomes. Box upper and lower borders correspond to the interquartile range (Q1–Q3). The horizontal lines within the boxes correspond to the median values and the diamond symbol to the mean values; the whisker limits indicate minimum and maximum observations after outlier removal. For biochemical and clinical pregnancy as well as for live birth rates, men who did not achieve a biochemical and clinical pregnancy and live birth were significantly older. For miscarriage, there was no difference. Pairs marked with an asterisk are statistically different

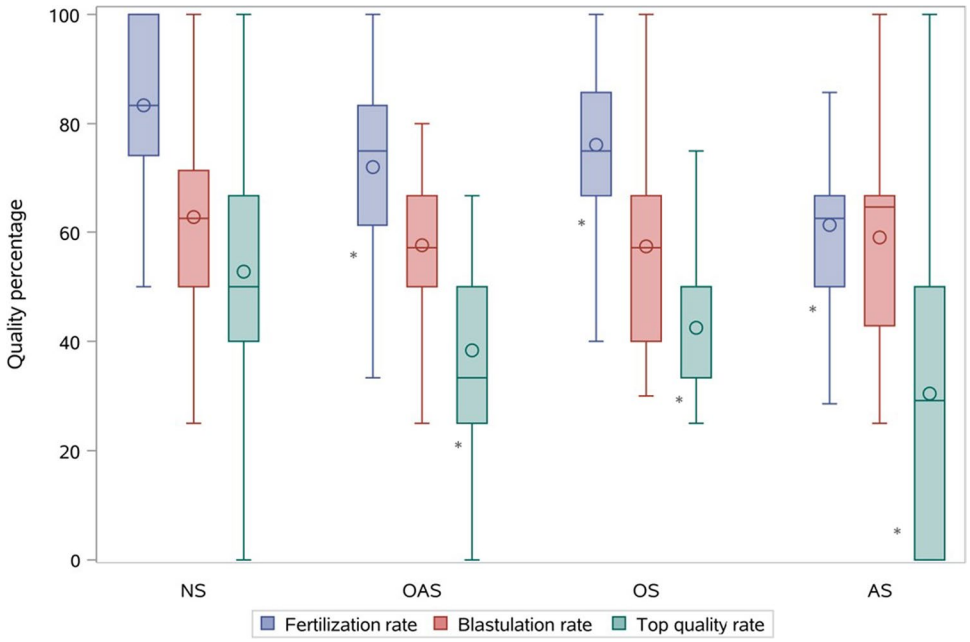

Fig. 2 Box and whisker plot of embryo quality parameters for the studied sperm groups. Box upper and lower borders correspond to the interquartile range (Q1–Q3). The horizontal lines within the boxes correspond to the median values and the circles to the mean values. The limits of the whiskers correspond to the minimum and maximum value without considering outliers. Cases marked with asterisk had statistically significant differences. NS, normozoospermia; OAS, oligoasthenozoospermia; OS, oligozoospermia; AS, asthenozoospermia

Table 3). When considering total motility as a semen quality parameter, analysis results indicate that this variable had no relation with any of the reproductive outcomes studied (all p values > 0.05).

In terms of clinical outcomes, differential results were observed for the investigated sperm parameters following OD-ICSI (Fig. 3). Specifically, the asthenozoospermic group presented reduced success rates in biochemical and clinical pregnancy, as well as in live birth with statistical significance ($p < 0.05$) (Table 3). Chi-square test demonstrated a twofold increase in the odds for a positive clinical outcome in the normozoospermic group, compared to the asthenozoospermic group ($p < 0.05$), although this result was not consistent for miscarriage (Supp. Table 4). Notably, OD-ICSI cycles conducted with asthenozoospermic samples had the lowest live birth rate compared to the other three groups

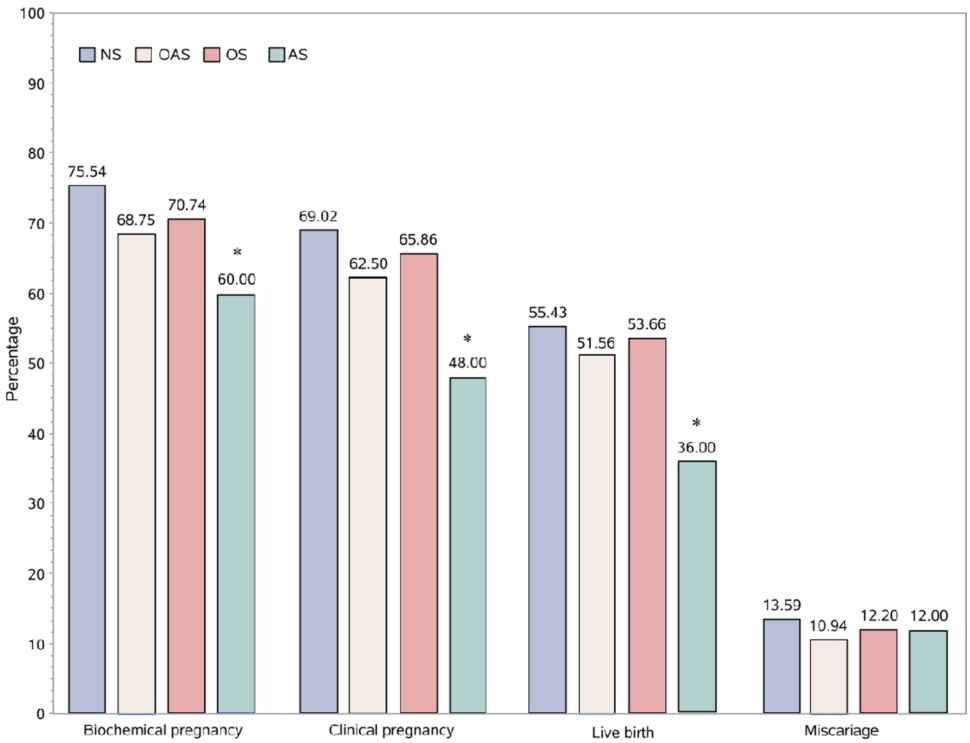

Fig. 3 Clinical outcomes and miscarriage rates following OD-ICSI for the different sperm parameter groups. Rates marked with an asterisk correspond to the statistically different outcomes of cycles with asthenozoospermia, as compared to normozoospermia. NS, normozoospermia; OAS, oligoasthenozoospermia; OS, oligozoospermia; AS, asthenozoospermia

Table 3 Contingency table of study groups and clinical outcomes following OD-ICSI. Each cell contains (from top to bottom) the number of cases, the row percentage, and the column percentage (in italics). NS, normozoospermia; OAS, oligoasthenozoospermia; OS, oligozoospermia; AS, asthenozoospermia

	Biochemical pregnancy	Clinical pregnancy	Live birth	Miscarriage	Negative β-hCG	Total
NS	139	127	102	25	45	184
	75.54%	69.02%	55.43%	13.59%	24.46%	54.28%
	57.44%	*58.26%*	*58.29%*	*58.14%*	*46.39%*	
OAS	44	40	33	7	20	64
	68.75%	62.50%	51.56%	10.94%	31.25%	18.88%
	18.18%	*18.35%*	*18.86%*	*16.28%*	*20.62%*	
OS	29	27	22	5	12	41
	70.74%	65.86%	53.66%	12.20%	29.27%	12.09%
	11.98%	*12.39%*	*12.57%*	*11.63%*	*12.37%*	
AS	30	24	18	6	20	50
	60.00%	48.00%	36.00%	12.00%	40.00%	14.75%
	12.40%	*11.01%*	*10.29%*	*13.95%*	*20.62%*	
Total	242	218	175	43	97	339
	71.39%	64.31%	51.62%	12.68%	28.61%	100.00%

combined ($p = 0.0167$; OR, 0.47; 95% CI, 0.25–0.88), while a negative serum β-hCG following ET was obtained in 40% of the cycles in the group, which was a significant outcome throughout ($p = 0.0296$; OR, 0.49; 95% CI, 0.25–0.93). Oligozoospermia and oligoasthenozoospermia did not present any significant differentiation in clinical outcomes when compared with normozoospermia ($p > 0.05$) (Supp. Table 4).

Logistic regression models for each individual reproductive outcome were constructed. The inputs were the male fertility status as stratified by sperm parameters and female age, with the aim to adjust the statistical findings for the latter. According to the results of the models generated, female age has an important role for biochemical pregnancy and live birth; however, asthenozoospermia is negatively related to clinical pregnancy, and more specifically, ART cycles with asthenozoospermia had about half the odds to achieve clinical pregnancy compared to cycles with normozoospermia (OR, 0.44; $p = 0.0368$), similarly for live birth (OR, 0.49; $p = 0.0425$) (Supp. Table 5, Supp. Fig. 2).

Discussion

The study evaluated the impact of male age and sperm quality parameters on the embryological and clinical outcomes of OD-ICSI cycles. A straightforward analysis indicated that progressive and total motility is affected by age, while an association of paternal age was demonstrated with fertilization rates, biochemical pregnancy, clinical pregnancy, and live birth. On the other hand, age appeared to be irrelevant with pregnancy loss and the progression to blastocyst as well as with the quality of the resulting embryos following ICSI. Comparisons and analysis based on the grouped sperm parameters on the differentiated male factors revealed that normal sperm parameters were more likely to result in a positive clinical outcome compared to any type of sperm quality abnormality. Sperm samples with reduced count and motility had lowered fertilization and top-quality embryo rates. Abnormalities solely linked with asthenozoospermia negatively affected implantation potential and advancement to clinical pregnancy and live birth, with significantly lowered rates throughout. Overall, total sperm count had a lesser, but considerable impact on fertilization and blastocyst development, while the achievement of a positive clinical outcome following OD-ICSI is strongly affected by male age and the percentage of spermatozoa with progressive motility. The profound significance of male age and sperm progressive motility in our outcomes may either suggest an age-related loss of progressive movement at advanced ages that was expressed in our study cohort or an equally important and independent presentation of each factor in the outcomes, considering the impeding effect of the "aging" sperm and the pathophysiology of asthenozoospermia. By discussing the outcomes of this study separately, we will attempt to provide a solid basis for further research to enhance our current knowledge on the "successful" sperm, as this will improve treatment strategies, prognosis of ART cycle outcomes, and personalized patient management.

A reduction in the number of spermatozoa in a given sample may relate to various conditions and environmental alterations, with the most common attribute of the effect being the probability of reduced chromatin quality and increased DNA damage that these sperm parameter groups are susceptible to [17, 37]. Total sperm count and sperm concentration have been previously investigated for their effect on embryological procedures in ART. While several studies revealed no effect of sperm concentration in ART outcomes [19, 23–27], others demonstrated a clear influence on fertilization [6, 17,

20, 21, 28, 30, 31], blastulation [6, 17, 21, 31], implantation rate [6], and day 1 embryo quality [20]. Our results are in accordance with the latter studies indicating a significant reduction in fertilization rates and affected embryo quality, although at different stages of embryo development.

Paternal age is currently being established in clinical practice as a surrogate factor in fertility evaluation, with emerging evidence denoting potentially detrimental effects on several aspects surrounding reproduction and a tendency of advanced age towards DNA mutations, chromosomal aberrations, and alterations of epigenetic patterns [38]. The effect of advanced male age has been extensively investigated with conflicting results, with several reports presenting no significant impact on clinical outcomes [39–42] in contrast to others demonstrating a negative effect on fertilization [6, 40], implantation, pregnancy, and live birth rates [6, 14, 43]. In an OD setting, our results agree with previous reports regarding the effect of age on fertilization, pregnancy, and live birth [6, 14, 40, 43] but with no difference regarding embryological parameters of blastulation and quality. While other studies denote a level of interference on early embryonic development [17, 21, 44] that was expected to be evident also in our study with the expression of paternal genes of older males impacting early embryos, this result could not be replicated. We anticipated statistically that such a result would have been significant if approximately 1000 cycles were to be included in the analysis. Since the clinical outcomes were indeed affected in the group of advanced male age, we must not exclude the possibility of a genetically stable contribution from the random population of male participants, skewing the results to normality rather than stressing the age effect on embryological aspects.

Semen volume and sperm motility are two characteristics that have previously been shown to deteriorate with increasing male age through a causal relationship [20, 22, 40, 45, 46]. We have demonstrated a negative correlation of advanced paternal age and sperm motility, with results reflecting a considerable effect on the clinical outcomes of asthenozoospermic males but not in the oligoasthenozoospermic group. By accounting that male age was statistically similar in both groups, we may either suggest that the asthenozoospermic group represents a loss in the motility function due to advanced age (possibly more evident in normally borderline motilities) or an autonomous pathological group that purely represents the impact of compromised motility in reproduction, distinct to that of oligoasthenozoospermia. In either case, new evidence is attentive to the importance of paternal age and progressive motility assessment in a formal and cohesive evaluation prior to any treatment and for the prognosis of its outcomes. Our clinical results indicate that ICSI treatment presents adequate results, comparable to those performed with normal sperm parameters, for male factor infertility of oligo- and oligoasthenozoospermia, while the age-related effect could not be reversed, and progressive motility importance was justified on the type of any reproductive treatment, even in ICSI, where is normally overtaken.

As an individual factor, progressive motility has been demonstrated to impact embryological and clinical outcomes following ICSI [6, 17, 20].Our study results are partly in agreement with the findings of Bartolacci et al. [17] and Borges et al. [20] confirming a negative correlation between motility and clinical outcomes post ICSI, although these authors did not adopt oocyte donation in their approach to restrict the effects of a female factor. The study by Setti et al. [6] employed donated — but previously vitrified — oocytes and reported an association between progressive motility and embryo development and implantation. Other studies that failed to demonstrate a direct association provided indications of a certain role of motility in the expected outcomes in ART. Specifically, Koppers et al. [18] indicated that ICSI cycles with established pregnancy had significantly better sperm motility patterns in the initial samples. In addition, Marriapen et al. [22] performed a regression analysis demonstrating a trend for poor sperm motility towards a lower chance of birth.

In an attempt to emphasize the importance of sperm motility in a controlled fertilization scheme such as in ICSI, it is essential to acknowledge the properties of the "slow" sperm. Reduced motility may reflect genetic conditions, ultrastructural and metabolic defects, lack of functional maturity, environmental factors in the epididymis, infections or inflammations of the male reproductive tract, oxidative stress, fragmented DNA, presence of antisperm antibodies, increased semen viscosity, or prolonged periods of anejaculation [47]. Asthenozoospermia has been associated with mutations in the dynein genes DNAI1, DNAH5, and DNAH11, with male carriers always exhibiting reduced sperm motility [48]. The proteomic signature of normozoospermic vs asthenozoospermic men is distinctly different, as at least 17 protein spots involved in crucial biological procedures such as axoneme activation, focal adhesion assembly, glycolysis, gluconeogenesis, cellular response to stress, and nucleosome assembly are negatively affected in asthenozoospermic patients [49, 50]. Described anomalies in the protein expression of asthenozoospermic samples fall into energy production, structure and movement, and cell signaling and regulation [49]. A direct link that justifies the potential effect of poor motility in ICSI, where sperm–oocyte interaction is overridden, is the fact that IMPA1 gene expression that promotes myo-inositol synthesis, which is essential for embryonic development, is affected in asthenozoospermic samples [49]. Motility alterations could be directly linked with Krebs cycle dysfunction [49], as the flagellar movement frequency is directly related to energy production from

ATP. The decreased energy production suggests a possible deregulation in the metabolic profile of asthenozoospermic men. Moreover, spontaneous acrosomal reaction negatively correlates with progressive sperm motility [51–53]. The acrosomal state and function are important aspects of gamete interaction during the fertilization process. Genes encoding for voltage-gated Na^+ channels have been shown to regulate sperm function, specifically, motility, hyperactivation, and acrosome reaction [54]. CatSper and CatSper2 genes code for proteins localized in the principal piece of the sperm flagellum, and targeted disruption of CatSper in knock-out mice markedly decreased motility, while sperm were unable to fertilize intact oocytes, and cAMP-mediated Ca2 + influx was abolished [55, 56]. In addition, a study by Collodel et al. [57] demonstrated that the aneuploidy rate assessed by means of fluorescence in situ hybridization (FISH) was higher in samples with reduced sperm motility, linking asthenozoospermia with an increased risk of chromosomally abnormal conceptions.

Evidence of the association between the degree of motility and the reproductive outcome emerge from multiple directions. Oxidative damage is increased in samples with lower sperm motility [58], while motility presents an inverse relationship with DNA fragmentation [59, 60]. Higher levels of DNA fragmentation, which may derive from oxidative stress, result in poorer ART outcomes [61, 62] and may contribute to recurrent pregnancy failures and increased incidence of miscarriage [63, 64]. In addition, metagenomic studies have demonstrated that the seminal microbiome is linked to alterations in sperm motility and an increase in DNA fragmentation as a result of inflammatory cytokines, overproduction of ROS, direct interaction of pathogens with sperm, or indirectly through the secretion of soluble factors [65, 66]. Thus, male populations presenting with poor sperm motility and disrupted microbiota could contribute genetic material, which is susceptible to breaks to the embryo, resulting in poorer prospects for the initiation and maintenance of a viable pregnancy.

In the context of an OD program, Capelouto et al. [19] were not able to identify any links with male age or sperm parameters and the reproductive outcomes; however, they presented a different setting that included variable sperm sources and methods of retrieval. Other studies that compare sperm motility with clinical results employed previous editions of the WHO guidelines [67] or other reference values that were not comparable to the ones employed in the current study [23–25, 28, 29]. Differences in the methodological approach of the aforementioned studies extend to several important aspects, as some studies analyzed ICSI and conventional in vitro fertilization (IVF) outcomes [28] or used computer-assisted semen analysis [25]. In addition, variable sperm sources and male infertility factors with severe forms were included in the analysis in some publications [24, 27, 28]. Overall, different populations with differently timed ETs and numbers of embryos transferred were described [23, 24].

The strengths of the current study mainly reside in the firm design, which allows the direct projection of the presented results on different male infertility types that are regularly met in the ART context. Oocyte donation offers improved clinical and obstetrical outcomes and represents an excellent scientific basis for the investigation of male parameters defocusing research from potential confounding factors of female infertility. The inclusion and exclusion criteria employed in this study engaged to reduce the cohort to only the representative cycles for the hypothesis, while consistency and uniformity were assured across the medical and embryological procedures and throughout the research methodology. To our knowledge and in the current scope, this study represents the most robust design to date by employing real-world data on distinct male fertility status groups. In addition, the study reports directly on both embryological and clinical outcomes with uniformity; by applying the WHO 2010 [9] lower reference values and specificity: by utilizing only oocyte donation cycles in the analyses to minimize potentially adverse oocyte contribution to the outcomes. Additionally, since male age has been previously implied to have a negative influence on these parameters, the concomitant analysis of this factor proved to be an essential complementary factor adding value to the study results. Overall, it is important to stress that procedural standardization and uniformity of procedures were warranted for all cycles by adequate and certified training of all medical and paramedical personnel, a strict set of standards in operating procedures implemented with internal and external audits and a stable scientific team that ensures familiarity and experience with the center procedures.

In terms of potential factors affecting oocyte donation success rates such as endometrial receptivity and recipient age, previously published data suggests that neither age nor previous diagnosis play a role in uterine receptivity [68, 69]. Albeit, in order to minimize a potential negative effect during the embryo implantation process and early pregnancy, the design of the current study excluded cases with a history of endometrial pathologies. On the other hand, although several studies support that the fertilization potential remains unaffected and identical to that of fresh sperm [70–72], sperm cryopreservation may also inflict an argued decrease in the quality of the samples. Moreover, a time-lapse study on morphokinetic parameters has revealed no differences between fresh and frozen ejaculated sperm used for ICSI as it excluded any effect of cryopreservation on the early key events of embryo development [73].

A possible limitation of the study is that strict assessment and comparison of sperm morphology was not performed; however, ICSI procedures were overall performed with morphologically compliant cells, and procedure

uniformity was present throughout the treatments. In addition and in support of this approach, previous evidence suggests that morphology related neither to ICSI outcome nor to the embryological and clinical outcomes [26]. A relatively small cohort size may be justified by the timing of initiation of OD treatment availability in the reporting fertility center and adequate cycle data for analysis, as also to the strict set of inclusion criteria that were employed to allow robust comparisons and a small fraction of the potentially eligible cycles that had incomplete follow-up. Other limitations of the study are mostly attributed to its retrospective nature and to the lack of adequate information about potential confounders such as smoking, BMI, and lifestyle aspects. Moreover, the potential factors surrounding the biochemical signaling of implantation and overall interaction between the embryo and the endometrium of the recipient are still scientifically vague and difficult to assess and quantify. In order to distinguish the origins of the presented motility alterations, the investigation of sperm DNA fragmentation in the samples included in the analysis would have been a valuable addition to the study as this would shed light on the origins of the presented motility alterations.

In conclusion, male age and progressive motility of spermatozoa are two variables that appear to have a strong influence on the outcomes of ART, even if motility is not challenged through ICSI procedures. There is a strong biological basis of the effect of both the "aged" and the "slow" sperm throughout the reproductive course that indefinitely positions these parameters as crucial candidates to consider when assessing male fertility.

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Author Contribution Paraskevi Vogiatzi conceived the study. The study was designed by Paraskevi Vogiatzi, Abraham Pouliakis, Maria Sakellariou, Aikaterini Athanasiou, and Adamantios Athanasiou. Data collection and analysis were performed by Paraskevi Vogiatzi, Abraham Pouliakis, Maria Sakellariou, Aikaterini Athanasiou, and Adamantios Athanasiou. The first draft of the manuscript was written by all authors, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability The data contained in this article will be shared on reasonable request to the corresponding author.

Code Availability Not applicable.

Declarations

Ethics Approval The study was approved by the Research and Ethics Committee of the Reproductive Center (EVD 0701/2020) and has been conducted in accordance with the ethical standards of the National Authority for Medically Assisted Reproduction and with the 1964 Helsinki Declaration and its later amendments.

Consent to Participate Throughout the study and especially during data acquisition and analysis, anonymity was strictly preserved by an initial assignment of an exclusive code for each participant, with no other identifying characteristics recorded. Patients received extensive consultation on the procedures to be followed, and written consent was obtained by all participants on treatment.

Consent for Publication Not applicable, no identifying information about participants is contained on the manuscript or in data collection and analysis performed.

Conflict of Interest The authors declare no competing interests.

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