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The impact of semen parameters on ICSI and pregnancy outcomes in egg recipient cycles with PGT-A

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Background

The egg donation model offers an opportunity to isolate the male factor and evaluate its impact on IVF-ICSI and pregnancy outcomes.

Objective

To study the effect of non-obstructive azoospermia (NOA) on ICSI and pregnancy outcomes compared with severe oligozoospermia (OS-S) and mild to moderate oligozoospermia (OS-MM) in egg recipient cycles.

Materials and methods

This is a retrospective longitudinal cohort study including 1,594 patients who underwent ICSI in egg recipient cycles with PGT-A. The cohort was divided into three groups: couples with NOA accounting for 479 patients (30%); couples with severe oligozoospermia (sperm number <5 x 10^6/ml),

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accounting for 442 patients (27.8%); couples with mild to moderate oligozoospermia, with sperm number >_5 x 10^6/ml and <15 x 10^6/ml, accounting for 673 patients (42.2%).

Results

The fertilization rate was significantly reduced in the NOA group as compared to the OS-S and the OS-MM group: 30.3% versus 63% and 77.3% (p<0.05). Logistic regression analysis adjusted for confounders highlighted NOA as a negative predictor of obtaining a euploid blastocyst both per injected oocyte and per obtained blastocyst. The miscarriage rate in the NOA group was 11.8%; higher than the OS-S and OS-MM groups (7% and 2.7%) (p<0.05). The live birth rate per ET was significantly lower in the NOA group compared to the OS-S and the OS-MM group (20.4% vs 30.3% and 35.4%, p<0.05). The risk of preterm labour was significantly higher in the NOA group, compared to the OS-S and OS-MM group (55.1% versus 46.8 and 16.1%, p<0.001) and this difference was observed in both singleton and twin pregnancies.

Discussion and conclusion

In our retrospective comparative study, non-obstructive azoospermia significantly affects early embryonic potential and live birth rates per cycle and per embryo transfer. It is also associated with higher risk of preterm birth. Future prospective multi-centre studies are needed to highlight the effect of sperm quality on ART and pregnancy outcomes.

Introduction

Surgical sperm retrieval techniques, such as testicular sperm aspiration (TESA), testicular sperm extraction (TESE) or micro-testicular sperm extraction (mTESE), have given a chance to couples in which the male partner was diagnosed with non-obstructive azoospermia (NOA) to father their genetically own child using intracytoplasmic sperm injection (ICSI). However, even nowadays, the percentage of couples with male infertility due to NOA who get to experience the birth of their genetically own child is still quite low (13.4%) [1]. In addition, the increased incidence of chromosomal problems in men with azoospermia and its possible correlation not only with chromosomally abnormal

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embryos [2] but also with adverse pregnancy outcomes has been shown by multiple studies [3,4]. Nevertheless, few studies [5] in the literature outline the impact of non-obstructive azoospermia in the subgroup of couples who opt to use donated eggs and they have included small numbers of azoospermic men.

The egg donation model offers an opportunity to isolate the male factor and evaluate its impact on IVF-ICSI and pregnancy outcomes, taking into account that the confounding factors of advanced maternal age, endometrial ageing and uterine factors cannot be fully evaluated. However, the effect of azoospermia on the euploidy rates of blastocysts and the prevalence of fetal and congenital anomalies can be estimated more accurately, when the oocytes are collected from young donors, reducing the confounding factor of poor oocyte quality.

Thus, the aim of our study was to study the effect of non-obstructive azoospermia (NOA) on the clinical outcome of ICSI in egg donor cycles compared with severe oligozoospermia (OS-S) and mild to moderate oligozoospermia (OS-MM), from ART parameters to obstetrical and perinatal outcomes. Our primary outcome was live birth rate which was defined as the percentage of all cycles that led to live birth per embryo transfer [6]. The analyses were performed per embryo transfer and per cycle to show the effect of male factor on the ART and clinical outcomes. Secondary outcomes included fertilisation rate, blastocyst formation rate, euploid blastocyst rate, positive pregnancy test rate, biochemical pregnancy loss rate, miscarriage rate, clinical pregnancy rate, preterm labour rate and mean birth weight.

Materials & methods

Study Population

This longitudinal cohort study involved 1,594 patients whose sperm was used for ICSI treatment using donated oocytes at the Reproductive Medicine Unit GENESIS Athens between January 2016 and May 2020. The indication for oocyte donation was history of severe female infertility, including premature ovarian insufficiency, low ovarian reserve, poor/low response to ovarian stimulation or recurrent unsuccessful IVF treatment.

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All anonymous oocyte donors were between 21 and 30 years of age and followed the criteria outlined by the American Society of Reproductive Medicine (ASRM)[7]. Recruitment was anonymous and altruistic as per national legislation. All donors were fit and well with no significant past medical history.

The cohort was categorized into three groups according to the male partner's sperm parameters: 1) couples with non-obstructive azoospermia (NOA), accounting for 479 patients (30%); 2) couples with severe oligozoospermia (OS-S) (sperm number <5 x 10^6/ml), accounting for 442 patients (27.8%); 3) couples with mild to moderate oligozoospermia (OS-MM), with sperm number >_ 5 x 10^6/ml and < 15 x 10^6/ml, accounting for 673 patients (42.2%).

While the term severe oligozoospermia is internationally recognized[8], we used the terms mild to moderate oligozoospermia to describe sperm concentrations between >_ 5 x 10^6/ml and < 15 x 10^6/ml for the purpose of our analysis.

All men received and completed a detailed questionnaire on the duration of infertility, previous pregnancies and their outcomes, andrological history and family history regarding infertility, recurrent miscarriages and children with congenital abnormalities.

Non-obstructive azoospermia was differentiated from obstructive azoospermia, based on physical exam, scrotal ultrasound, hormonal and genetic studies. Physical exam and scrotal ultrasound were used to calculate testicular volume and to exclude the presence of testicular tumours, unilateral or bilateral absence of the vas deferens, epididymal head or tail dilatation and varicocele. Specifically, men with an FSH above 8 mIU/ml and atrophic testes (longitudinal axis less than 5cm), along with no genital tract obstruction, were deemed to have non-obstructive azoospermia and were counselled towards surgical sperm retrieval[8]. In cases when it was needed to extract sperm by testicular sperm extraction (TESE), a specimen was also sent for histopathological analysis showing varying degrees of impaired spermatogenesis, based on the Johnsen score[9].

Patients with obstructive azoospermia due to iatrogenic vasal injury (e.g., previous pelvic surgery or trauma) were excluded, as were patients with congenital bilateral absence of the vas deferens, as most have normal spermatogenesis [10].

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Genetic analysis was performed for every male patient, including karyotype and Y-chromosome microdeletions. Preimplantation genetic testing for aneuploidies (PGT-A) was performed for severe male factor infertility, previous history of repeated implantation failure or pregnancy loss or a combination of these conditions. Cycles involving PGT for monogenic disease, patients with obstructive azoospermia, including patients with cystic fibrosis, and cycles with NOA in which sperm could not be recovered by surgical sperm extraction were excluded from this study.

The oocyte fertilisation rate was calculated by the total number of fertilized oocytes divided by the number of injected oocytes. Fourteen days after the embryo transfer a serum HCG test was performed. A positive pregnancy test was defined as HCG levels above 25 mIU/ml (Roche Diagnostic International, Switzerland). Biochemical pregnancy was defined as the decline of hCG level after a positive pregnancy test and the absence of an identifiable pregnancy on ultrasound. Clinical pregnancy was defined by the presence of an intrauterine gestational sac documented by transvaginal ultrasound examination 5 weeks after the embryo transfer. Pregnancy loss between 8 and 20 weeks of gestation was considered as miscarriage. Preterm birth was defined as birth between the 20th and the 37th week of gestation. Low birth weight infants were defined as $\leq$ 2500g. All pregnancies were followed up until miscarriage or delivery.

Karyotype and Y chromosome analysis

Peripheral blood lymphocyte karyotype analysis was performed in all male patients. Blood was collected in heparin vacutainer tubes (Becton Dickinson, USA). Karyotypes were examined using conventional G-banding techniques. No less than 25 metaphase spreads were inspected for each case and up to 100 metaphases in cases of mosaicism with a minimal resolution of 550-band per haploid chromosome set. Karyotype description was as per the International System for Human Cytogenetic Nomenclature [11].

Y chromosome microdeletions (YCMs) were confirmed with a multiplex polymerase chain reaction (PCR), using sequence-tagged sites (STS), in the entire AZF region. This protocol follows the recommendations from the EEA/EMQN guidelines [12].

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Sperm analysis

After 2 to 5 days of sexual abstinence, semen samples were collected after masturbation and analysed according to the WHO guidelines [13].

In the case of NOA, sperm was collected initially with testicular aspiration (TESA). Testicular sperm extraction (TESE) was used when no spermatozoa were obtained with TESA.

In the TESA procedure a standard butterfly needle was used connected to a 10 ml syringe in order to facilitate manual aspiration. The testicle was punctured with the needle which was steadily moved in different directions after negative pressure was created, to obtain an adequate sample. The same technique was also performed in the other testicle if the sample was insufficient from one side. In the event that the TESA technique did not provide any motile spermatozoa the patient underwent TESE. The TESE procedure was performed by making a one-site or multiple-site incision at the same or contralateral testis and excising a small amount of testicular tissue. In cases when TESE was needed, a sample was also sent for histopathological examination.

In this cohort of men with NOA, sperm was recovered by means of TESE in 197 cases, while in the other 282 it was recovered by bilateral TESA.

Fresh sperm was used in all cases. In cases of TESA, the aspirated sperm sample was washed by centrifugation on QUINN'S ADVANTAGE Medium with HEPES (SAGE) and the suspension moved to a Petri dish to check for the presence of spermatozoa. In cases of TESE fresh testicular tissue was dissected using needles in a Petri dish and subsequently inspected under the microscope. The tissue samples were preserved using the same medium.

Fresh given sperm and suspensions were treated with graded density fractions (Pure Sperm 100, NIDACON) and washed by centrifugation on QUINN'S ADVANTAGE Medium with HEPES (SAGE).

Oocyte and Embryo handling procedures

Ovarian stimulation for the egg donors was performed following a short flare GnRH agonist (Arvekap 0.1mg, Ipsen, France) stimulation protocol starting on the second day of the menstrual cycle with recombinant FSH (follitropin alpha) (Gonal-F; MERCK) starting on the third day of the menstrual cycle.

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When one or more follicles have reached a mean diameter of 18-20mm, induction of oocyte maturation was performed, using a subcutaneous injection of 250mg β-hCG (Ovitrelle*, MERCK). The transvaginal oocyte retrieval was performed 36 hours after the administration of β-hCG. MII oocytes were selected for immediate vitrification. The Cryotop method for oocyte vitrification described by Kuwayama et al [14] was used. The vitrification and warming solutions were acquired from Kitazato. Oocytes were equilibrated after collection at room temperature for 15 min in the equilibrium solution (ES) (7.5% (v/v) ethylene glycol (EG) with 7.5% dimethylsulfoxide (DMSO) in TCM199 medium and 20% synthetic serum substitute (SSS)). They were subsequently placed in the vitrification solution (VS) (15% EG with 15% DMSO and 0.5 M sucrose). After one minute they were set on the cryotop strip and submerged into liquid nitrogen (LN). The vitrified oocytes were kept in quarantine for a minimum period of 6 months. Storage of the oocytes was achieved in vapour tanks (V1500-AB Isothermal Freezer, CBS).

To achieve warming, the cryotop was extracted from the LN and immediately transferred in 1.0 M sucrose with TCM199 and 20% SSS at 37°C. After 1 min, oocytes were placed in 0.5 M sucrose in M199 and 20% SSS at room temperature for 3 min. Finally, one five-minute wash followed by a one-minute wash was performed with TCM199 + 20% SSS at room temperature. Standard culture conditions were used for 2 hours for the surviving oocytes before ICSI.

Injected oocytes were incubated in 20 µL drops of 1-STEP (SAGE, Trumbull, USA) until evaluation of fertilisation. 16-18 hours after ICSI the presence of two pronuclei was confirmed and embryos were placed in fresh 1-STEP (SAGE, Trumbull, USA) under standard culture conditions until they reach the blastocyst stage (D5-D6). The quality of blastocysts was assessed according to the criteria of Gardner and Schoolcraft [15].

PGT-A method

120-170 hours after injection, blastocysts were evaluated according to their degree of expansion and the quality of inner cell mass (ICM) and trophectoderm cells. Blastocysts selected for embryo transfer were subjected to trophectoderm biopsy. In brief, using a series of 2 to 3 pulses on a non-contact laser (LYKOS, Hamilton-Thorne Biosciences, Beverly, USA), a small hole in the zona pellucida was opened opposite the inner cell mass and blastocysts were incubated for a further 2 hours (approximately) to allow trophectoderm cell herniation. At the time of biopsy, blastocysts were placed individually in a

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dish prepared with 3 droplets of 10µl of Quinn's advantage medium enriched with HEPES and 5% Human Serum Albumin (SAGE, Trumbull, USA), overlaid with pre-equilibrated mineral oil for tissue culture (SAGE, Trumbull, USA) on a heated stage of an Olympus IX71 microscope, equipped with micromanipulation tools and a diode laser (LYKOS, Hamilton-Thorne Biosciences, Beverly, USA). Each blastocyst was placed on the holding pipette and positioned so the ICM was discernible and opposite the biopsy pipette (Cook Medical, USA). The trophectoderm cells (5-10) were gently aspirated with moderate suction into the biopsy pipette while synchronously firing 2 to 3 laser pulses aimed at the thinnest junctions between trophectoderm cells and stretching them gently to separate them from the blastocyst proper. Following the biopsy procedure, the blastocyst was placed in culture medium and vitrified until the PGT-A report. The retrieved biopsied trophectoderm cells were stored in -80C until further use. Comprehensive chromosome testing was performed by means of CGH-ARRAY (Agilent). Euploid blastocyst transfer was performed during a frozen-thawed cycle. Our management followed local legislation indicating that up to two euploid embryos may be transferred, unless only one euploid embryo was identified after PGT-A. Supernumerary euploid embryos remain frozen for future use.

Endometrial preparation for embryo transfer

The endometrial preparation protocol of the egg recipients involved the administration of oral estradiol valerate (Cyclacur®, Bayer) starting from 2mg twice a day and titrated appropriately until the endometrial thickness reached 8mm with a trilaminar pattern. The progesterone supplementation dose was 400 mg intravaginal capsule twice a day five days prior to the embryo transfer up to 12 weeks of gestation.

Statistical analysis

Continuous data are presented as absolute values, mean +/- SD. Categorical variables are presented as absolute values, percentage and 95% confidence intervals (CI). Differences in variables between the different groups of couples with NOA, OS-S and OS-MM and the clinical impact on IVF and reproductive outcome, were statistically analysed with chi-squared test. Multivariable logistic

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regression models were developed to calculate unadjusted and adjusted odds ratios (aOR) and 95% confidence intervals (CI) and then compared with use of Analysis of Variance (ANOVA). Multivariable modelling was conducted using logistic regression adjusting for AZF deletions, abnormal karyotypes and male partner's age as covariates and fertilisation rate, euploid blastocyst formation rate and live birth rate as dependent outcomes. R software version 2.14.2 (Free Software Foundation) was used for statistics and logistic regression analyses. For all statistical comparisons, significance was deemed if p was <0.05. A post hoc power analysis was conducted at http:// powerandsamplesize.com.

Ethics approval

The study was approved by the Medical Board of Aretaieio Hospital, the Ethics Committee of the National and Kapodistrian University of Athens and the Review Board of the GENESIS Reproductive Medicine Unit.

Results

A total of 1,594 ICSI couples were included in the study. The participants were divided into three groups, depending on the semen analysis results, as described in the methods section. Baseline characteristics are described in Table 1. There was no statistical difference in the age of male participants or the age of egg recipients. Male FSH levels were significantly higher and testosterone levels significantly reduced in the OS-S and NOA group compared to the OS-MM group ($p<0.0001$). The abnormal karyotypes were also statistically significantly higher ($p<0.05$) in the NOA group compared to the OS-S and OS-MM group and the same result was seen when looking at the Y chromosome microdeletions and AZF deletions specifically.

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Table 1. Epidemiological data - parameters of sperm and infertility issues in infertile men & women, classified in groups with mild to moderate oligozoospermia, severe oligozoospermia and non-obstructive azoospermia

Epidemiological data	A MILD TO MODERATE OLIGOZOOSPERMIA (OS-MM) (n=673)	B SEVERE OLIGOZOOSPERMIA (OS-S) (n-442)	C NON-OBSTRUCTIVE AZOOSPERMIA (NOA) (n=479)
Age of male partner (years)	45.02 ± 3.03	45.29 ± 2.64	44.11 ± 3.28
Age of female partner (years)	41.28 ± 2.56	41.67 ± 2.32	40.91 ± 2.22
FSH (mIU/ml)	6.73 ± 2.99 [b,c]	12.64 ± 3.79 [a,c]	32.33 ± 6.22 [a,b]
Free testosterone (pg/dl)	12.92 ± 2.29 [b,c]	9.03 ± 3.06 [a,c]	6.17 ± 4.35 [a,b]
Abnormal karyotypes	3.4% (23/673) [b,c]	6.6% (29/442) [a,c]	12.7% (61/479) [a,b]
AZF deletions	2.7% (18/673) [b,c]	10.6% (47/442) [a,c]	17.1% (82/479) [a,b]

a: statistically significantly different to group A; b: statistically significantly different to group B; c: statistically significantly different to group C

Prevalence of chromosomal abnormalities

The prevalence of chromosomal abnormalities was higher in the NOA group, 12.7% (61/479, 95% CI 9.9-16.1%), than in the OS-S group, 6.6% (29/442, 95% CI 4.4-9.3%, p<.001) and the OS-MM group,

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3.4% (23/673, 95% CI 2.2-5 %, p<0.001) (Table 1). Klinefelter syndrome accounted for 42.5% (48/113, 95% CI 33.2-52.1%) of all chromosomal abnormalities and its prevalence was higher in the NOA group, 6.7% (32/479, 95% CI 4.6-9.3%), than in the OS-S group, 2.3% (10/442, 1.1-4.1%, p<0.001) or the OS-MM group, 0.8% (6/673, 95% CI 0.3-1.9%, p<0.001) (Supplemental Table 1). All individual chromosomal abnormalities found are presented in Supplemental Table 1.

Prevalence of AZF deletions

Overall, we found an AZF deletion in 17.1% (82/479, 95% CI 13.8-20.8%) of subfertile patients in the NOA group, significantly higher than in the OS-S group, 10.6% (47/442, 95% CI 7.9-13.9%, p<0.05) and in the OS-MM group, 2.7% (18/673, 95% CI 3.3-6.7%, p<0.05) (Table 1). In all three groups, the majority of AZF deletions were AZFc deletions, where spermatogenesis is possible. In the NOA group, 85.3% were isolated AZFc deletions, while in the OS-S group and the OS-MM group it was 91.5% and 88.9% respectively. All individual Y chromosome microdeletions are presented in Supplemental Table 2.

ART outcomes

There was no difference in the number of injected MII oocytes between the three groups: 13.05 +/- 1.93 (n = 8,788), 13.07 +/- 2.01 (n = 5778), 13.19 +/- 2.1 (n = 6319), respectively, in OS-MM, OS-S and NOA (table 2).

A total of 12,349 normally fertilized MII oocytes were obtained. The mean number of zygotes in the NOA group was significantly lower versus both the OS-S and the OS-MM group (P<0.001, Table 2) 4 +/- 1.54 (n=1917), 8.23 +/- 2.21 (n=3639), 10.09 +/- 1.95 (n=6793) in NOA, OS-S and OS-MM, respectively. Subsequently, the fertilization rate was significantly reduced in the NOA group compared to the OS-S and OS-MM group: 30.3% versus 63% and 77.3% respectively (P<0.05, Table 2).

In addition, there was a statistically significant lower mean number of blastocysts obtained in the NOA group versus the OS-S and OS-MM group (P>0.0001, Table 2) (n=5,801 in total): 1.43 +/- 0.52 (n=808), 2.63 +/- 1.12 (n=1178), 5.67 +/- 2.07 (n=3815) in NOA, OS-S and OS-MM, respectively. The blastocyst

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formation rate per injected MII oocyte was significantly reduced in the NOA group compared to the OS-S and OS-MM group: 13%, versus 20.4% and 43.4% respectively (P<0.0001, Table 2). However, this difference for the NOA group disappeared compared to the OS-S group when calculated per fertilised oocyte (42.1% vs 32.4%). No difference was reported in blastocyst morphology among the different groups (Supplemental Figure 1).

Similarly, the mean number of euploid blastocysts was also significantly lower in the NOA group compared to the OS-S and OS-MM group (P<0.05, Table 2): 1.11 +/- 0.38 (n=531), 1.80 +/- 1.69 (n=812), 3.83 +/- 1.11 (n=2575) in NOA, OS-S and OS-MM, respectively. Indeed, the euploid blastocyst rate per injected MII oocyte was significantly reduced in NOA (8.4%) compared with both OS-S (14%) and OS-MM (29.3%) (P<0.001, Table 2). However, the euploidy rate per biopsied blastocyst was similar among the three study groups (65.7%, 68.9% and 67.5%; Table 2).

Table 2. ART outcomes

Fertilization Outcome/ Embryo development	**A MILD TO MODERATE OLIGOZOOSPERMIA (OS-MM) (n=673)**	**B SEVERE OLIGOZOOSPERMIA (OS-S) (n-442)**	**C NON-OBSTRUCTIVE AZOOSPERMIA (NOA) (n=479)**	**Total**
MII oocytes injected, n (mean +/- SD)	8788 (13.05 ± 1.93)	5778 (13.07 ± 2.01)	6319 (13.19 ± 2.10)	20885
2PN fertilised oocytes, n (mean +/- SD)	6793 (10.09 ± 1.95) [b,c]	3639 (8.23 ± 2.21) [a,c]	1917 (4.00 ± 1.54) [a,b]	19142
2PN fertilised/MII oocytes injected, n, % (95% CI)	6793/8788	3639/5778 63% (61.8 – 64.2) [a,c]	1917/6319	12349/20885

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	77.3% (76.4 – 78.2) b,c		30.3% (29.2 – 31.5) a,b	59.1% (58.5-59.8)
Blastocysts, n (mean +/- SD)	3815 (5.67 ± 2.07) b,c	1178 (2.63 ± 1.12) a,c	808 (1.43 ± 0.52) a,b	9616
Blastocysts/MII oocytes injected, n, % (95% CI)	3815/8788 43.4% (42.4 – 44.4) b,c	1178/5778 20.4% (19.4 – 21.4) a,c	808/6319 13% (12.2 – 13.8) a,b	5801/20885 27.8% (27.1-28.4)
Blastocysts/ 2PN fertilized oocytes, n, % (95% CI)	3815/6793 56.2% (55-57.4) b,c	1178/3639 32.4% (30.9-33.9) a,c	808/1917 42.1% (39.9-44.3) a	5801/12349 47% (46.1-47.9)
Euploid blastocysts, n (mean +/- SD)	2575 3.83 ± 1.11 b,c	812 1.80 ± 1.69 a,c	531 1.11 ± 0.38 a,b	3918
Euploid Blastocysts/MII oocytes injected, n, % (95% CI)	2575/8788 29.3% (28.3 - 30.3) b,c	812/5778 14% (13.1 – 14.9) a,c	531/6319 8.4% (7.7 – 9.1) a,b	3918/20885 18.8% (18.2-19.3)
Euploid Blastocysts/blastocysts, n, % (95% CI)	2575/3815 67.5% (66 – 69)	812/1178 68.9% (66.3 – 71.5)	531/808 65.7% (62.4 – 69)	3918/5801 67.5% (66.3-68.7)
Cancelled Cycles (no euploid blastocysts), n, % (95% CI)	1 /673 b,c 0.14% (0 - 0.4)	9/442 a 2% (0.7 – 3.3)	18/479 a 3.7% (2 – 5.4)	28/ 1594 1.8% (1.2-2.5)

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Clinical outcomes

The number of ETs performed was 1,566: 672, 433 and 461 in OS-MM, OS-S and NOA, respectively. Overall, 32.7% of the ETs performed were single ET (n = 513/1,566), and the rate was significantly higher in the NOA group compared to OS-S and OS-MM (84.8% versus 26.1% and 1.3%, p<0.0001) (Table 3), due to <2 available euploid blastocysts in most cycles. The positive pregnancy test rates per ET were 42%, 42.7% and 41.6% in OS-MM, OS-S and NOA, respectively. There was a higher biochemical pregnancy loss rate in the NOA group compared to the OS-S and OS-MM group (29.2% versus 15.1% and 8.9%; p<0.05) (Table 3). The same finding was noted, when looking at the miscarriage rates, when the miscarriage rate in the NOA group was 11.8%, higher than the OS-S and OS-MM group (7% and 2.7%, respectively; P<0.05) (Table 3). The clinical pregnancy rate was similar in the OS-MM and OS-S group, but significantly lower in the NOA group (38.2% versus 36.3% and 29.5% respectively; P<0.05) (Table 3). The total number of babies born during the period of our study was 463. The live birth rate per ET was statistically lower in the NOA group compared to the OS-S and the OS-MM group (20.4% vs 30.3% and 35.4%; P<0.05) (Table 3). The live birth rate was also statistically lower in the OS-S group compared to the OS-MM group (P<0.05).

Cycle outcomes

There was a significantly higher cancelled cycle rate due to no euploid blastocysts in the NOA group compared to the OS-MM group (3.7% vs 0.14%, p<0.05) (Table 2). However, there was no difference between the NOA and OS-S group (3.7% vs 2%) (Table 4). The effect of male factor was still significant both for the live birth and clinical pregnancy rates, when the analysis is performed per cycle, with significantly lower rates for the NOA group, compared to the other two groups (Table 3).

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Table 3. Clinical outcomes

Reproductive outcome by male factor	A MILD TO MODERATE OLIGOZOOSPERMIA (OS-MM) (N=673)	B SEVERE OLIGOZOOSPERMIA (OS-S) (n-442)	C NON-OBSTRUCTIVE AZOOSPERMIA (NOA) (N=479)	Total
ET, n	672	433	461	1566
Single ET, n (%)	9/672 (1.3%) [b,c]	113/433 (26.1%) [a,c]	391/461 (84.8%) [a,b]	513/1566 (32.7%)
Double ET, n (%)	663/672 (98.7%) [b,c]	320/433 (73.9%) [a,c]	70/461 (15.2%) [a,b]	1053/1566 (67.2%)
Positive hCG/ET, n, % (95% CI)	282/672 42% (38.3-45.7)	185/433 42.7% (38 – 47.4)	192/461 41.6% (37.1 – 46.1)	659/1566 42.1% (39.6-44.6)
Positive hCG/Cycle, n, % (95% CI)	282/673 41.9% (38.2-45.7)	185/442 41.8% (37.2-46.7)	192/479 40% (35.7-44.6)	659/1594 41.3% (38.9-43.8)
Biochemical pregnancy loss/positive hCG, n, % (95% CI)	25/282[b, c] 8.9% (5.6-12.2)	28/185[a, c] 15.1% (9.9-20.3)	56/192[a.b] 29.2% (22.8-35.6)	109/659 16.5% (13.8-19.6)

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Miscarriages/clinical pregnancies, n, % (95% CI)	7/257[c] 2.7% (0.7 – 4.7)	11/157[c] 7% (3 – 11)	16/136[a, b] 11.8% (6.4 – 17.2)	34/550 6.2% (4.3-8.5)
Clinical Pregnancy/ET, n, % (95% CI)	257/672 38.2% (34.5-41.9)	157/433 36.3% (31.8-40.8)	136/461 [a,b] 29.5% (25.3-33.7)	550/1566 35.1% (32.8-37.5)
Clinical Pregnancy/Cycle, n, % (95% CI)	257/673 38.2% (34.5-41.2)	157/442 35.5% (31.1-40.2)	136/479 [a.b] 28.4% (24.4-32.7)	550/1594
Live Birth/ ET, n, % (95% CI)	238/672 35.4% (31.8 – 39)	131/433 30.3% (26-34.6)	94/461[a, b] 20.4% (16.7 – 24.1)	463/1566 29.6% (27.3-31.9)
Live Birth/ Cycle, n, % (95% CI)	238/673 35.4% (31.8-39.1)	131/442 29.6 (25.4-34.1)	94/479 [a.b] 19.6% (16.2-23.5)	463/1594 29.1% (26.8-31.3)

a: statistically significantly different to group A; b: statistically significantly different to group B; c: statistically significantly different to group C

Perinatal and obstetric outcomes

There was an increased rate of preterm labour in the NOA group, compared to the OS-S and OS-MM group (61.7% versus 45.8% and 17.6%; p<0.001) (Table 4) (Supplemental figure 2). The mean gestational age at birth in the NOA group was 33.94 (+/- 3.97) weeks, which was lower compared to

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the OS-S group (36.8+/- 2.09) and the OS-MM group (37.82 +/-1.99) (Table 4). When looking at the singleton pregnancies the risk of preterm labour was significantly higher in the NOA group, compared to the OS -S and OS-MM group (55.1% versus 46.8 and 16.1%; p<0.05) (Table 5). In twin pregnancies, the risk of preterm labour was again higher in the NOA group compared the OS-S and OS-MM group (87.5% versus 43.2 and 21.9%, p<0.001) (Table 5). Similarly, in terms of mean birth weight, there was a significantly higher number of babies born with a birth weight <2.5 kg from the NOA group, compared to the OS-S and OS-MM group (34% versus 19.8% and 12.2%, p<0.001) (table 4) (Supplemental figure 3). In twenty (3.6%) of the 550 clinical pregnancies there was a major congenital malformation diagnosed that led to the termination of pregnancy (eight cardiac anomalies, four cerebral anomalies, two genitourinary anomalies, three spinal cord anomaly and one gastrointestinal anomaly).

Table 4. Obstetric outcomes

Reproductive outcome by male factor	A MILD TO MODERATE OLIGOZOOSPERMIA **(**OS-MM**)** (n=238)	B SEVERE OLIGOZOOSPERMIA (OS-S) (n=131)	C NON-OBSTRUCTIVE AZOOSPERMIA (NOA) (n=94)	Total
Preterm labor/Live birth	42/238 17.6% (13-23.1)	60/131 [a] 45.8% (37.1-54.7)	58/94 [a,b] 61.7% (51.1-71.5)	160/463 34.6% (30.2-39.1)
Low birth weight (<2.5Kg)/live birth	29/238 12.2% (8-17)	26/131 [a] 19.9% (13.4-27.8)	32/94 [a,b] 34% (24.6-44.6)	87/463

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				18.8% (15.3-22.7)
Mean gestational age at labour (weeks)	37.82 ± 1.99 [b,c]	36.8 ± 2.09 [a,c]	33.94 ± 3.97 [a,b]	
Mean Birth weight neonates (g)	3289.31 ±463.22 [b,c]	2972.77 ±606.47 [a,c]	2863.5 ±413.59 [a,b]	

a: statistically significantly different to group A; b: statistically significantly different to group B; c: statistically significantly different to group C

Table 5. Preterm labor in singleton and twin pregnancies

Preterm labor	OS-MM	OS-S	NOA	Total
Singleton pregnancies	28/174 16.1% (10.6-21.6)	44/94[a] 46.8% (36.1-56.3)	43/78[a,b] 55.1% (44.1-66.1)	115/346 33.2% (28.2-38.4)
Twin pregnancies	14/64 21.9% (11.7-31.9)	16/37[a] 43.2% (27.2-59.2)	14/16[a,b] 87.5% (69.9-100)	44/117 37.6% (28.8-47)

a: statistically significantly different to group A; b: statistically significantly different to group B; c: statistically significantly different to group C

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Logistic Regression Analyses and Post Hoc Power Calculation

Three multivariable regression analyses were conducted to assess whether possible confounders can affect the likelihood to have at least a 50% fertilisation rate per cycle, the likelihood to obtain a euploid blastocyst per cycle and the likelihood to achieve a live birth per cycle (Supplemental table 3). Having an abnormal karyotype was shown to be a significant predictor of both fertilisation rate (OR 0.24; 95% CI, 0.16-0.35, P<0.001) and euploid blastocyst rate (OR 0.70; 95% CI, 0.66-0.74, P<0.001).) (Supplemental table 3). When comparing the effect of types of abnormal karyotype to the possible semen analysis results, only Klinefelter syndrome appeared to be statistically significantly correlated with NOA (OR= 4.62; 95% CI, 3.30-19.19, P<0.00001). Male age also affected significantly the fertilization rate (OR 0.97; 95% CI, 0.949-0.996, P: 0.024) and euploid blastocyst rate (OR 0.99; 95% CI, 0.989-0.997, P<0.001) (Supplemental table 3). In contrast, AZF deletions did not have a statistically significant effect on any of the outcomes (Supplemental table 3).

Post-hoc power analysis

The primary endpoint of the study for the post-hoc analysis was the euploid blastocyst rate. When the euploidy rate per injected oocyte was 29.3% in the mild to moderate oligozoospermia group, employing the number of euploid blastocysts per group as the population, the post hoc power calculation indicated a 0.99 statistical power to exclude a 2% difference.

Discussion

This study aimed to assess the impact of NOA in egg donor cycles in order to reduce the confounding factor of poor oocyte quality. All cycles with NOA were compared to cycles with OS-S and OS-MM to evaluate whether there is any effect on the successful outcome of ICSI cycles.

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We found that deteriorating semen parameters had a significant impact in lowering fertilization rates, blastocyst formation rates and euploid blastocyst rates. While surgically retrieved sperm from men with NOA has been shown to have impaired fertility potential [16-19], our study elucidated that sperm coming from a man with OS-S also affects significantly ART outcomes compared to men with OS-MM. Our study's findings agreed with other studies [20,21] that sperm quality can have a significant effect on ICSI outcomes and euploid blastocyst rate, albeit these studies did not focus on egg donor cycles.

While Capelouto et al [5] supported that semen parameters do not significantly affect IVF/ICSI outcomes in a vitrified oocyte donation model, it only included 27 men with a sperm count of less than 6 mil/ml. Our study contains data from a large cohort of subfertile men who are either azoospermic or eligible for ICSI, representing an unselected population of males visiting one of the largest tertiary referral centers in Greece. The main strength of our study is the number of patients we could include in each individual group, allowing more accurate comparisons into the effect of male factor. We also controlled for the potential confounding factors of paternal and maternal age. However, it needs to be noted that our population was significantly older than in other similar studies, with most men being >45 years old and the majority of egg recipients being >40 years old. While some studies [22-24] have shown that paternal age can affect reproductive outcomes in oocyte-donor models, some others [25] have shown that ICSI can overcome any potential negative effects. In our study, albeit advanced paternal age might have contributed to the low rates of fertilization and euploid blastocyst formation, particularly in the NOA group, it is illustrated that paternal age on its own does not explain the difference in outcomes between the groups.

A noticeable finding in our study is the significantly lower fertilization rate in the NOA group, as the main contributor to the lower transferrable blastocyst formation rate per injected oocyte, compared to the other two groups. This was despite the fact that only fresh sperm was used in our study, which is associated with better fertilisation rates than frozen-thawed sperm [26]. The cause of this effect seems to be dual. Testicular spermatozoa, on one hand, have a lower competence for fertilisation since the ultimate steps of sperm maturation take place in the epididymis [26,27], while they also reveal higher rates of chromosomal aneuploidy [28,29]. While other studies [30,31] have reported higher fertilisation rates when testicular sperm is used from patients with non-obstructive azoospermia, our study only included patients that either had varying degrees of impaired spermatogenesis, maturation arrest or tubular sclerosis on histopathology or significantly high FSH levels and testicular atrophy that are

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correlated with defective spermatogenesis [4]. Some authors advocate that sperm activation methods might improve outcomes in this group [32], however, there is still insufficient evidence to support their widespread use.

Regarding the clinical outcomes, while there was no difference in the implantation rates, there was a significantly higher miscarriage rate, lower clinical pregnancy rate and lower live birth rate per cycle in the NOA group compared to the other two groups. This finding was quite interesting as previous studies [33] have indicated that while semen parameters might influence the ART outcomes, usually the effect disappears after implantation has occurred. Mazilli et al[33] conducted a retrospective analysis on 1,219 consecutive ICSI cycles combined with PGT-A and concluded that while severe male factor subfertility can adversely affect early embryonic competence, the euploidy rate and implantation potential of the obtained blastocysts remain independent from sperm quality. However, that study was underpowered to evaluate clinical outcomes in the group of azoospermic men and it focused on ICSI cycles using autologous oocytes when egg quality can be a confounding factor.

To investigate further the reason why there is a lower euploid blastocyst rate and live birth rates in the NOA group, we assessed the effect of Y chromosome microdeletions and chromosomal abnormalities on ART and clinical outcomes. Our data suggest that AZF deletions, the majority of which were AZFc deletions, are not an independent predictor of fertilisation rate, euploid blastocyst and live birth rates. Although the effect of AZF deletions on fertilisation rate and embryo quality has been demonstrated in many studies [34-36], the literature has been contentious on whether they affect miscarriage rates and live birth rates. The findings of our study have disagreed with some previous studies [37,38] and agreed with others [39-41.] Given this continuous inconsistency of results, a prospective multi-center study would provide further clarity as to whether these outcomes are affected in AZF-deleted men compared to AZF-intact men.

Chromosomal abnormalities in azoospermic and oligoasthenospermic men have been associated with miscarriage, especially in translocations and pericentric inversions [42,43]. In our study ICSI treatment in men with a chromosomal abnormality was associated with significantly lower fertilisation and euploid blastocyst rates, compared to men with a normal karyotype, but there was no effect on live birth rate. Notably, in our study, most chromosomal abnormalities were gonosomal and in particular Klinefelter's, with only a minority being translocations and inversions in which PGT-A has been shown to reduce miscarriage rate [44]. This finding adds to the controversy as to whether PGT-A should be

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offered to men with Klinefelter syndrome [45], although newer technologies such as next-generation sequencing (NGS) might provide additional benefits in this subgroup of patients [44]. In general, though, more data are required from future studies to draw a clear conclusion on these still controversial issues, particularly focusing on specific chromosomal anomalies and their effect on pregnancy outcomes.

The risk of preterm labour has been shown to be increased in donor oocyte recipients when compared to autologous patients [46] and the effect is even more pronounced in twin pregnancies [47]. The exact reason has not been clarified, although higher incidence of gestational hypertension [48] and pre-eclampsia [49,50] has been speculated to play a significant role. In our study, preterm labour rates both for singleton and twin pregnancies were significantly higher in the NOA and the OS-S group, compared to the OS-MM group, suggesting that sperm quality can also have an important contribution to preterm labour and lead to lower mean birth weights, with the effect persisting when focusing on singleton pregnancies. A major limitation of our study is that we were not able to control for obstetric complications, such as pre-eclampsia, which would result in medically indicated delivery. Importantly, the women in our study had a mean age of over 40 years and are already at higher risk of preeclampsia than younger women [51]. Thus, using donor oocytes might increase the likelihood of preeclampsia even further, possibly accounting for the high rates of preterm labour in our study. The link between sperm quality and preterm labour is less clear. A Swedish population study [52] showed that the risk for preeclampsia can be attributed to paternal factors in 13% of cases, highlighting genetic interactions with maternal genetic factors as a possible cause. Other researchers indicated that HLA-G variants deriving from the father might produce a paternal-fetal susceptibility component than can predispose to preeclampsia [53]. In addition, raised chance of pre-eclampsia and small-for-gestational-age (SGA) babies in primigravidas with short duration of sperm exposure from their partners prior to pregnancy has been reported [54,55]. Based on that hypothesis, given male partners with azoospermia have no sperm cells in their seminal fluid, their female partners will be unable to generate protective immunity against preeclampsia. Finally, some studies have shown that ICSI pregnancies from azoospermic and oligospermic partners have an increased risk of developing preeclampsia [56,57], whereas a more recent study [58] that looked at pregnancy outcomes in subfertile population needing ART treatment and divided them based on aetiology of subfertility, found that pregnancies in the male infertility group had a higher incidence of SGA but there was no association with preterm labour. The relationship between sperm quality, preterm delivery, and preeclampsia warrants further exploration.

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Conclusion

In our retrospective comparative study looking at 1,594 patients, non-obstructive azoospermia significantly affects early embryonic potential and live birth rates per cycle. It is also associated with a higher risk of preterm birth. Future prospective multi-centre studies are needed to highlight the effect of sperm quality on clinical and pregnancy outcomes.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare

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None

AUTHOR CONTRIBUTIONS

Alexandros Grammatis designed the study, acquired data, helped with data analysis and wrote the manuscript. Athanasios Pappas acquired data, helped with the data analysis and editing of the review. Georgia Kokkali and Kostas Pantos helped with the conception of the study, the acquisition of data and editing of the review. Nikos Vlahos made substantial editorial amendments to the review.

All authors read the draft manuscript and made intellectual contributions to the final version.

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