Effect of the male factor on the clinical outcome of intracytoplasmic sperm injection combined with preimplantation aneuploidy testing: observational longitudinal cohort study of 1,219 consecutive cycles

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Objective: To evaluate the impact of the male factor on the outcomes of intracytoplasmic sperm injection (ICSI) cycles combined with preimplantation genetic testing for aneuploidies (PGT-A).

Design: Observational longitudinal cohort study.

Setting: Private in vitro fertilization (IVF) center.

Patient(s): A total of 1,219 oocyte retrievals divided into five study groups according to sperm parameters: normozoospermia (N), moderate male factor (MMF), severe oligoasthenoteratozoospermia (OAT-S), obstructive azoospermia (OA), and nonobstructive azoospermia (NOA).

Intervention(s): ICSI with ejaculated/surgically retrieved sperm, blastocyst culture, trophectoderm-based quantitative polymerase chain reaction PGT-A, and frozen-warmed euploid embryo transfer (ET).

Main Outcomes Measure(s): The primary outcome measures were fertilization, blastocyst development, and euploidy rates; the secondary outcome measures were live birth and miscarriage rates. Perinatal and obstetrical outcomes were monitored as well.

Result(s): A total of 9,042 metaphase II oocytes were inseminated. The fertilization rate was significantly reduced in MMF, OAT-S, OA, and NOA compared with N (74.8%, 68.7%, 67.3%, and 53.1% vs. 77.2%). The blastocyst rate per fertilized oocyte was significantly reduced in MMF and NOA compared with N (48.6% and 40.6% vs. 49.3%). The timing of blastocyst development also was affected in OA and NOA. Logistic regression analysis adjusted for confounders highlighted NOA as a negative predictor of obtaining an euploid blastocyst per OPU (odds ratio 0.5). When the analysis was performed per obtained blastocyst, however, no correlation between male factor and euploidy rate was observed. Embryo transfers also resulted in similar live birth and miscarriage rates. No impact of sperm factor on obstetrical/perinatal outcomes was observed.

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Conclusion(s): Severe male factor impairs early embryonic competence in terms of fertilization rate and developmental potential. However, the euploidy rate and implantation potential of the obtained blastocysts are independent from sperm quality. (Fertil Steril® 2017;108:961–72. ©2017 by American Society for Reproductive Medicine.)

Key Words: Preimplantation genetic testing, euploid blastocyst, aneuploidy, male factor, azoospermia

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The recent development of assisted reproductive technologies (ART) has allowed in vitro fertilization for many men with severe oligoasthenoteratozoospermia (OAT-S) or azoospermia who were previously excluded from in vitro fertilization (IVF) treatments (1–3). Today, preimplantation genetic diagnosis (PGD) with the use of sophisticated molecular technologies (quantitative polymerase chain reaction [qPCR], single-nucleotide polymorphism array, comparative genome hybridization [aCGH], next-generation sequencing) have been introduced in ART (4–10). PGD is indicated to circumvent the transmission of many known genetic and chromosomal conditions in the parental genotype to the offspring (11–13). It is also well known that both the aneuploidy rate at the blastocyst stage and a successful IVF outcome are strongly correlated with maternal biologic age (14–16). For this reason, preimplantation genetics is increasingly promoted for aneuploidy testing (preimplantation genetic testing for aneuploidies [PGT-A]), especially because of the increased age of the female patient population (17, 18).

Unfortunately, a general international consensus on the indication for PGT-A is still missing. According to Harper et al. (19), indications for aneuploidy testing are: 1) advanced maternal age (AMA, defined as maternal age >37 y); 2) repeated implantation failures (RIF: three or more failed transfers of good-quality embryos); 3) recurrent pregnancy loss (RPL; three or more previous miscarriages); and 4) severe male factor (SMF). SMF remains debatable; Lee et al. (4) limited the indications to only the first three, excluding SMF. Although the literature addresses PGT-A in AMA, the age threshold to consider a woman to be of AMA is still under discussion. In contrast to PGT-A for AMA, there is a lack of scientific data on the application of PGT-A in patients with SMF, which includes azoospermia (both obstructive and nonobstructive), OAT-S, macrocephalic sperm, Klinefelter syndrome, Y-chromosome microdeletion, and even men whose semen analysis does not fulfil the current World Health Organization (WHO) criteria in general.

Contradictory data are reported on the consequences of male-factor infertility on IVF-ICSI outcomes, embryo developmental competence, and incidence of embryo aneuploidy. Regarding the impact of male age, the data are sparse and discordant, and there is not a definite cutoff to consider “advanced paternal age” as an indication for PGT-A. Moreover, there is currently no definition of “advanced paternal age”; most authors considered $\geq$35 years (20–23), and others $\geq$40 years (24, 25) and $\geq$50 years (26).

Certain lifestyle behaviors, such as smoking habits, alcohol and drug consumption, and (mis)use of anabolic drugs, can cause a significant decrease in fertility potential in the couple. But the real impact of these lifestyle habits on ICSI outcome remains unclear (27–29). An increased body mass index (BMI) can cause a significant reduction of the number and motility of spermatozoa (30–32), but its effects on ICSI outcomes remain controversial (30, 33–36).

An increased DNA fragmentation rate can be related to a higher miscarriage rate (37), but some authors warned that it can not be considered to be an independent risk factor for reduced fertilization rate, embryo quality, and pregnancy rate (38, 39).

Finally, other authors (40, 41) showed that SMF may contribute to a higher prevalence of aneuploid embryos in ART. However, this conclusion is mainly based on studies using fluorescence in situ hybridization (FISH) analysis for a limited number of chromosomes on cleavage-stage embryos.

The aim of the present study was therefore to evaluate the impact of the male factor, in terms of sperm parameters, age, BMI, and smoking, on ART outcomes, i.e., fertilization, blastocyst formation, euploidy, and pregnancy rates. Preliminary obstetrical and perinatal outcomes were monitored as well.

MATERIALS AND METHODS

Study Population

This observational longitudinal cohort study involved 1,219 consecutive ICSI cycles performed for 1,090 couples at Genera Center–Clinica Valle Giulia in Rome from April 2013 to December 2015 with the use of qPCR-based PGT-A performed at the blastocyst stage. One year of observation and pregnancy follow-up was included in the study.

The cohort was divided into five groups according to the male partner’s sperm parameters, based on the WHO criteria (2010): 1) couples with normozoospermic (N) male partners, accounting for 528 of the 1,219 cycles (43.3%); 2) couples with moderate male factor (MMF), with sperm number $\geq 5 \times 10^6$/mL and $<15 \times 10^6$/mL, accounting for 420 cycles (34.5%); 3) couples with OAT-S (sperm number $<5 \times 10^6$/mL) or cryptozoospermia, accounting for 188 cycles (15.4%); 4) couples affected by obstructive azoospermia (OA), accounting for 34 cycles (2.8%); and 5) couples affected by secretory or nonobstructive azoospermia (NOA), accounting for 49 cycles (4.0%).

Genetic analysis was performed for every patient: karyotype, research of CFTR gene mutations, and, in case of azoospermia, Y-chromosome microdeletions. PGT-A was proposed because of: 1) AMA, here defined as $\geq$35 years; 2) RIF; 3) RPL; or 4) a combination of these conditions.

Sperm and egg donation cycles, cycles with severe female factor (alterations of the karyotype, other genetic alterations, uterine malformations, severe endometriosis, autoimmune diseases, BMI >30 kg/m^2 or <18.5 kg/m^2), cycles involving PGD for monogenic disease or chromosomal abnormalities, and cycles with NOA in which spermatozoa were not recovered by means of testicular sperm extraction (TESE; n = 52/101; 51.5%) were excluded from this study.

Sperm Handling Procedures

Each couple gave written informed consent to the treatment. The Institutional Review Board of the clinic approved this retrospective study.

After 3–5 days of sexual abstinence, semen samples were collected by masturbation and analyzed according to WHO (2010) guidelines.

In cases of OA, spermatozoa were recovered by means of testicular fine-needle aspiration (FNA-TESA), or surgically by means of TESE in case of NOA or when no spermatozoa were obtained after FNA-TESA (1, 42).

For TESE, freshly retrieved tissue was washed with the use of buffered medium (HEPES supplemented with human serum albumin; Sage In-Vitro Fertilization), then it was disrupted with the use of two glass slides or fine needles to obtain a suspension of small pieces. The suspension was then centrifuged at 300*g* for 8–10 minutes, and the pellet was inspected under the inverted microscope to assess the presence of sperm. For FNA-TESA, the suspension was washed with the use of HEPES-buffered medium, then centrifuged at 300*g* for 8–10 min, and the pellet was inspected under the inverted microscope to assess the presence of sperm.

Oocyte and Embryo Handling Procedures

In female partners, follicular stimulation was performed using the protocol with recombinant FSH and GnRH antagonist as previously described (18, 43). Oocyte pick-up (OPU) was performed 35–36 hours after induction of final oocyte maturation via transvaginal ultrasound–guided aspiration.

After oocyte retrieval, cumulus-oocyte complexes were exposed to 40 IU/mL hyaluronidase solution in HEPES-buffered medium (Sage In-Vitro Fertilization). Then the cumulus was removed mechanically with the use of plastic pipettes (denuding pipettes; Cook) on a heated-stage stereomicroscope under a hood (IVF Tech); insemination of oocytes by means of ICSI was carried out immediately after denudation, as previously described (44).

Each inseminated oocyte was placed in 25 μL culture medium (Quinn Advantage Cleavage Medium [Sage In-Vitro Fertilization] supplemented with 5% human serum albumin, or Continuous Single Culture Media [Irvine Scientific]) covered by prewarmed mineral oil (Sage In-Vitro Fertilization) and incubated in 6% CO_2 and 5% O_2 tension. In case Quinn Advantage medium was used, a changeover of new preequilibrated medium (Quinn Advantage Blastocyst Medium [Sage In-Vitro Fertilization] supplemented with 5% human serum albumin) was performed on day 3.

Fertilization was assessed 16–18 hours after ICSI. Oocytes displaying two pronuclei and a second polar body were considered to be normally fertilized and cultured further.

The blastocyst expansion grade and quality of the inner cell mass (number of cells and degree of compaction) and trophectoderm cells (number and dimension of the cells and morphologic appearance) were evaluated as reported previously (45).

Preimplantation Genetic Testing for Aneuploidies

At 120–168 hours after insemination, selected fully expanded blastocysts underwent trophectoderm biopsy as previously described (45). Briefly, all biopsy procedures were carried out on a heated stage in 10 μL HEPES-buffered medium overlaid with prewarmed mineral oil. A diode laser (Research Instruments) was used to open a 10–20-μm hole in the zona pellucida and cut the selected trophectoderm fragment (5–10 cells) which was aspirated into the biopsy pipette (Research Instruments).

After biopsy, all blastocysts were immediately vitrified (Cryotop device and solutions; Kitazato Biopharma Co.), according to the method described by Cobo et al. (46).

Chromosome analysis of trophectoderm cells were performed at Genetyx (Marostica). Comprehensive chromosomal testing was performed by means of qPCR: A multiplex amplification of 96 loci was carried out, and a method of relative quantification was adopted as previously described (6, 7). A karyotype prediction of the copy number status of each chromosome for each embryo was made by a certified cytogeneticist.

Euploid blastocyst transfers were all performed during a warming cycle. A single-embryo transfer (ET) was offered in the presence of euploid blastocyst(s), and it was performed as previously described (18).

A positive pregnancy test was defined as two increasing values of hCG level by >50 mIU/mL (47). The decline of hCG level after a positive test and the absence of an identifiable pregnancy on ultrasound examination were defined as biochemical pregnancy. Clinical pregnancy was determined by ultrasound demonstration of a gestational sac at 7 weeks. Spontaneous termination of pregnancy from week 7 to week 20 was considered to be a miscarriage (18). The miscarriage rate was assessed as the number of pregnancy losses (<20 gestational weeks) per clinical pregnancy achieved.

Perinatal outcomes were defined as birth weight and gestational age. Congenital malformations were considered to be conditions that caused functional impairment and/or required surgical correction.

Basal and cycle characteristics were recorded (female and male age, FSH, LH, antimüllerian hormone [AMH], and T levels, smoking habits, BMI, main infertility factor, duration of infertility, number of previous miscarriages and/or IVF failures, indication for PGT-A, numbers of metaphase II (MII) oocytes retrieved, zygotes, blastocysts, and euploid blastocysts, culture medium, blastocyst morphology, and day of biopsy).

TABLE 1

Cycle and embryologic outcomes by male factor.

Outcome	N	MMF	OAT-S	OA	NOA	Total
OPU, n	528	420	188	34	49	1,219
Female age, y, mean ± SD (range)	39.9 ± 3.0 (23.1–44.1)	39.7 ± 3.0 (25.6–44.0)	38.9 ± 3.3 (28.4–44.0)[a]	37.9 ± 2.9 (31.9–42.0)[a]	36.6 ± 4.9 (26.4–44.0)[a]	39.5 ± 3.2 (23.1–44.1)
MII oocytes inseminated, n (mean ± SD; range)	3,492 (6.6 ± 4.0; 1–22)	3,166 (7.5 ± 4.4; 1–24)[a]	1,653 (8.8 ± 5.1; 1–26)[a]	281 (8.3 ± 6.0; 1–33)	450 (9.2 ± 4.9; 1–22)[a]	9,042 (7.4 ± 4.5; 1–33)
Fertilized oocytes, n (mean ± SD; range)	2,696 (5.1 ± 3.3; 0–18)	2,367 (5.6 ± 3.5; 0–19)	1,136 (6.0 ± 3.9; 0–19)[a]	189 (5.6 ± 4.4; 0–23)	239 (4.9 ± 3.9; 0–17)	6,627 (5.4 ± 3.6; 0–23)
Fertilized/MII oocytes inseminated, n, % (95% CI)	2,696/3,492 77.2% (75.8–78.6)	2,367/3,166 74.8% (73.2–76.3)[a]	1,136/1,653 68.7% (66.5–70.9)[a]	189/281 67.3% (61.6–72.5)[a]	239/450 53.1% (48.5–57.7)[a]	6,627/9,042 73.3% (72.4–74.2)
Cycles without fertilized oocytes, n, % (95% CI)	4/528 0.8% (0.2–2.1)	9/420 2.1% (1.1–4.2)	2/188 1.1% (0.2–4.0)	2/34 5.9% (1.0–21.0)[a]	3/49 6.1% (1.6–17.9)[a]	20/1,219 (1.6%, 1.0–2.6)
Blastocysts, n (mean ± SD; range)	1,329 (2.5 ± 2.1; 0–11)	1,151 (2.7 ± 2.1; 0–13)	545 (2.9 ± 2.4; 0–14)	86 (2.5 ± 2.1; 0–9)	97 (2.0 ± 1.9; 0–9)	3,208 (2.6 ± 2.1; 0–14)
Blastocysts/MII oocytes inseminated, n, % (95% CI)	1,329/3,492 38.1% (36.5–39.7)	1,151/3,166 36.4% (34.7–38.1)	545/1,653 33.0% (30.8–35.3)[a]	86/281 30.6% (25.5–36.2)[a]	97/450 21.6% (18–25.6)[a]	3,208/9,042 35.5% (34.5–36.5)
Blastocysts/fertilized oocytes, n, % (95% CI)	1,329/2,696 49.3% (47.4–51.2)	1,151/2,367 48.6% (46.6–50.6)	545/1,136 48.0% (45.1–50.9)	86/189 45.5% (38.6–52.6)	97/239 40.6% (34.6–46.9)[a]	3,208/6,627 48.4% (47.2–49.6)
Cycles without blastocysts, n, % (95% CI)	50/528 9.5% (7.2–12.4)	36/420 8.6% (6.2–11.8)	9/188 4.8% (2.4–9.2)	6/34 17.6% (7.4–35.2)	12/49 24.5% (13.8–39.2)[a]	113/1,219 9.3% (7.7–11.1)
Euploid blastocysts, n (mean ± SD; range)	570 (1.1 ± 1.3; 0–7)	531 (1.3 ± 1.4; 0–7)	248 (1.3 ± 1.6; 0–11)	45 (1.3 ± 1.5; 0–6)	50 (1.0 ± 1.6; 0–8)	1,444 (1.2 ± 1.4; 0–11)
Euploid/MII oocytes inseminated, n, % (95% CI)	570/3,492 16.3% (15.1–17.6)	531/3,166 16.8% (15.5–18.1)	248/1,653 15.0% (13.3–16.8)	45/281 16.0% (12.2–20.8)	50/450 11.1% (8.5–14.4)[a]	1,444/9,042 16.0% (15.2–16.7)
Euploid/blastocysts, n, % (95% CI)	570/1,329 42.9% (40.3–45.6)	531/1,151 46.1% (42.3–49.0)	248/545 45.5% (41.4–49.7)	45/86 52.3% (41.9–62.6)	50/97 51.6% (41.7–61.2)	1,444/3,208 45.0% (43.3–46.7)
Cycles without euploid blastocysts, n, % (95% CI)	244/528 46.2% (42.0–50.1)	159/420 37.9% (33.2–42.7)[a]	67/188 35.6% (28.9–43.0)[a]	11/34 32.4% (18.0–50.6)	25/49 51.0% (36.5–65.4)	506/1,219 41.5% (38.7–44.4)

Note: CI = confidence interval; MII = metaphase II; MMF = moderate male factor; N = normozoospermic; OAT-S = severe oligoasthenoteratozoospermia; OA = obstructive azoospermia; NOA = nonobstructive azoospermia; OPU = oocyte pick-up.

[a] *P*< .05 versus N.

Mazzilli. Male factor and outcomes in PGT-A cycles. Fertil Steril 2017.

Statistical Analysis

Cycle data were collected in a relational database (Fertillab). Continuous data are presented as absolute values, mean ± SD, and range. Categoric variables are presented as absolute values, percentage, and 95% confidence interval (CI). Fisher exact test and paired *t* test were used to assess differences between categoric and continuous variables, respectively. A *P* value of <.05 was considered to be significant.

A logistic regression analysis was done to evaluate potential confounders among basal and cycle characteristics on the likelihood of a couple to obtain a transferable blastocyst per OPU and on the likelihood of each single blastocyst to be euploid. R software version 2.14.2 (Free Software Foundation) was used for statistics and logistic regression analyses. A post hoc power analysis was conducted at http://powerandsamplesize.com.

RESULTS

A total of 1,219 cycles performed for 1,090 couples were included in this study (Supplemental Tables 1 and 2 and Supplemental Figs. 1 and 2 are available online at www.fertstert.org). There was no significant difference in duration of infertility, number of previous pregnancy losses, number of previous IVF failures, female baseline hormonal levels and BMI, and male age and BMI among the patients included in the different arms of the study (Supplemental Table 1). The mean female age was significantly reduced in OAT-S, OA, and NOA compared with N (38.9 ± 3.3, 37.9 ± 2.9, 36.6 ± 4.9, and 39.9 ± 3.0, respectively; *P*<.01; Supplemental Table 1). Male FSH levels were significantly higher and T levels significantly reduced in OAT-S and NOA compared with N (*P*<.001; Supplemental Table 1).

Overall Embryologic Outcomes

A total of 9,042 MII oocytes were inseminated. The number of MII oocytes collected per cycle was on average higher in all of the study groups versus N: 6.6 ± 4.0 (n = 3,492), 7.5 ± 4.4 (n = 3,166), 8.8 ± 5.1 (n = 1,653), 8.3 ± 6.0 (n = 281), and 9.2 ± 4.9 (n = 450), respectively, in N, MMF, OAT-S, OA, and NOA (*P*<.001; Table 1).

A total of 6,627 normally fertilized MII oocytes were obtained, but only the OAT-S group showed a significantly higher mean number of zygotes versus N (*P*<.05; Table 1): 5.1 ± 3.3 (n = 2,696), 5.6 ± 3.5 (n = 2,367), 6.0 ± 3.9 (n = 1,136), 5.6 ± 4.4 (n = 189), and 4.9 ± 3.9 (n = 239) in N, MMF, OAT-S, OA, and NOA, respectively. Indeed, the fertilization rate was significantly reduced in MMF, OAT-S, OA, and NOA compared with N: 74.8%, 68.7%, 67.3%, and 53.1% versus 77.2% (*P*<.05; Table 1).

Further on along preimplantation development, there was no statistically significant difference in the mean number of blastocysts obtained among the five study groups (n = 3,208 in total): 2.5 ± 2.1 (n = 1,329), 2.7 ± 2.1 (n = 1,151), 2.9 ± 2.4 (n = 545), 2.5 ± 2.1 (n = 86), and 2.0 ± 1.9 (n = 97) in N, MMF, OAT-S, OA, and NOA, respectively. Again, the blastocyst rate per inseminated MII oocyte was significantly reduced in OAT-S, OA, and NOA compared with N: 33.0%, 30.6%, and 21.6% versus 38.1%; *P*<.05; Table 1). For the NOA group, it was significantly lower than in N even if calculated per fertilized oocyte (40.6% vs. 49.3%); No difference was reported in blastocyst morphology among the different groups, although the embryos in the OA and NOA groups had a significantly longer timing of development to the blastocyst stage (and were therefore more often biopsied later) than the embryos in the N group (*P*<.01; Supplemental Fig. 1).

The mean number of euploid blastocysts was similar in the 5 study groups: 1.1 ± 1.3 (n = 570), 1.3 ± 1.4 (n = 531), 1.3 ± 1.6 (n = 248), 1.3 ± 1.5 (n = 45), and (1.0 ± 1.6 (n = 50) in N, MMF, OAT-S, OA, and NOA, respectively. In fact, the euploid blastocyst rate per inseminated MII oocytes was significantly reduced only in NOA (11.1%) compared with N (16.3%; *P*<.01). Notably instead, the euploidy rate per biopsied blastocyst was similar among the five study groups (42.9%, 46.1%, 45.5%, 52.3%, and 51.6%; Table 1).

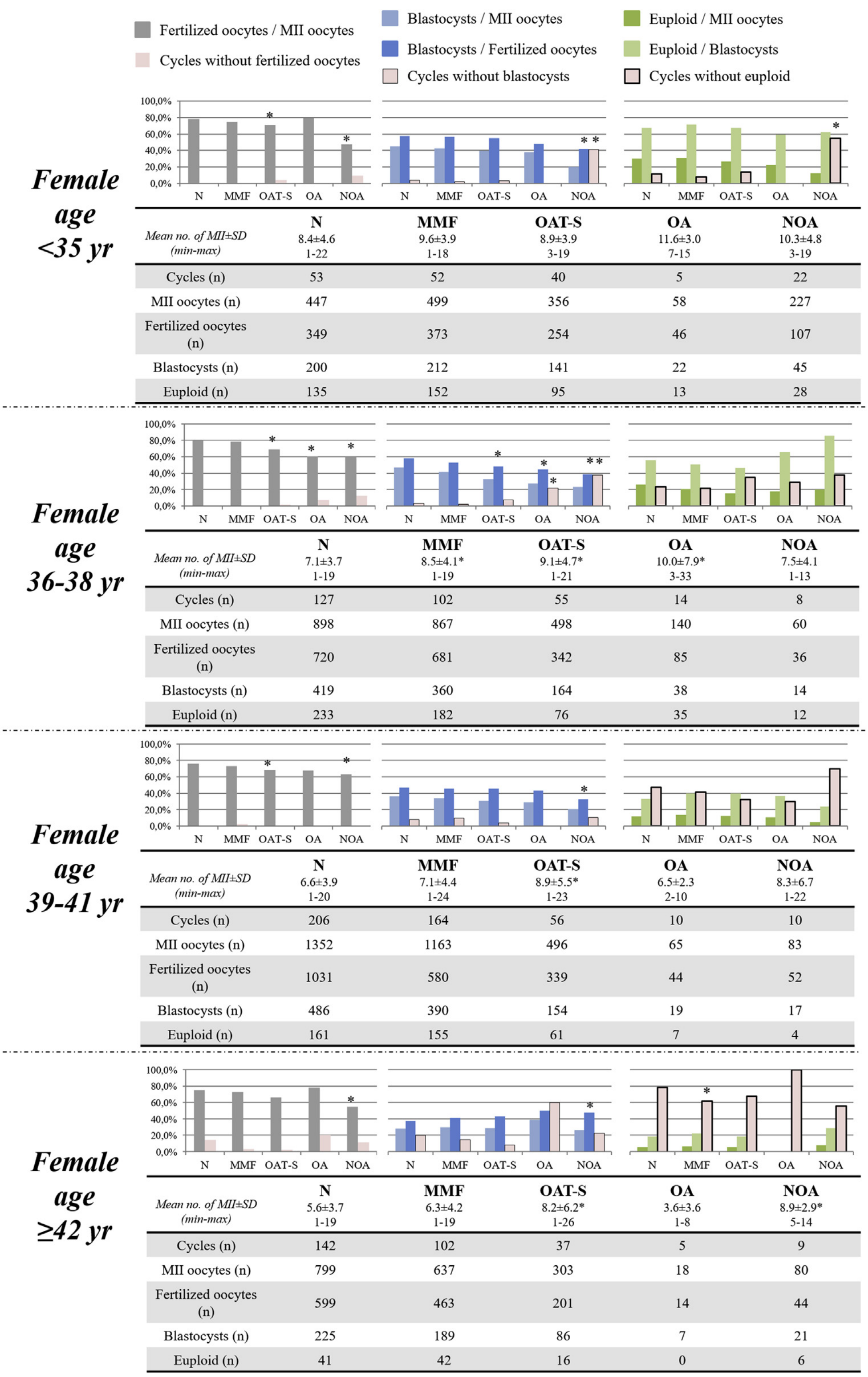
Overall Cycle Outcomes

The rates of cycles without fertilized oocytes were higher in OA and NOA versus N already from the overall analysis (5.9% and 6.1% vs. 0.8%; *P*<.05; Table 1). However, further on along preimplantation development, the rate of cycles without blastocysts was significantly higher only in NOA versus N (24.5% vs. 9.5%; *P*<.05; Table 1). Interestingly, no significant difference was reported in the rate of cycles without euploid blastocysts produced when comparing the azoospermic groups together with N. However, that rate was significantly lower in OAT-S compared with N (35.6% vs. 46.2%; *P*<.05; Table 1). Such outcomes may be ascribable to a younger population of women in the SMF groups, with a better prognosis per se which counterbalanced the negative effect the male factor in the initial phases of preimplantation development (Table 1). Therefore, to provide a more homogeneous analysis of the results, we performed two subanalyses according to female age at OPU and number of MII oocytes collected.

The female partners in the SMF study groups were characterized by a better prognosis due to both generally lower age at OPU and higher number of MII oocytes collected compared with N (*P*<.05; Supplemental Figs. 2A and 2B). Nonetheless, azoospermic couples overall underwent a higher number of PGT-A cycles in the study period (*P*<.05; Supplemental Fig. 2C). Figures 1 and 2 show the results sub-divided by ranges of female age at OPU and ranges of MII oocytes collected.

Figure 1 displays the results in the five study groups according to ranges of female age at OPU (<35, 36–38, 39–41, and ≥42 years). Importantly, the trends already highlighted by the overall results were here confirmed. Indeed, the SMF groups, especially NOA, showed generally lower fertilization and blastocyst rates than N, but no differences were reported in the euploidy rates calculated per either MII oocyte inseminated or blastocyst obtained. On a per-cycle–based analysis, this translates to a rate of cycles conducted for NOA couples in which no euploid blastocyst was produced

FIGURE 1



Female age <35 yr

	N	**MMF**	**OAT-S**	**OA**	**NOA**
Mean no. of MII±SD (min-max)	8.4±4.6 1-22	9.6±3.9 1-18	8.9±3.9 3-19	11.6±3.0 7-15	10.3±4.8 3-19
Cycles (n)	53	52	40	5	22
MII oocytes (n)	447	499	356	58	227
Fertilized oocytes (n)	349	373	254	46	107
Blastocysts (n)	200	212	141	22	45
Euploid (n)	135	152	95	13	28

Female age 36-38 yr

	N	**MMF**	**OAT-S**	**OA**	**NOA**
Mean no. of MII±SD (min-max)	7.1±3.7 1-19	8.5±4.1* 1-19	9.1±4.7* 1-21	10.0±7.9* 3-33	7.5±4.1 1-13
Cycles (n)	127	102	55	14	8
MII oocytes (n)	898	867	498	140	60
Fertilized oocytes (n)	720	681	342	85	36
Blastocysts (n)	419	360	164	38	14
Euploid (n)	233	182	76	35	12

Female age 39-41 yr

	N	**MMF**	**OAT-S**	**OA**	**NOA**
Mean no. of MII±SD (min-max)	6.6±3.9 1-20	7.1±4.4 1-24	8.9±5.5* 1-23	6.5±2.3 2-10	8.3±6.7 1-22
Cycles (n)	206	164	56	10	10
MII oocytes (n)	1352	1163	496	65	83
Fertilized oocytes (n)	1031	580	339	44	52
Blastocysts (n)	486	390	154	19	17
Euploid (n)	161	155	61	7	4

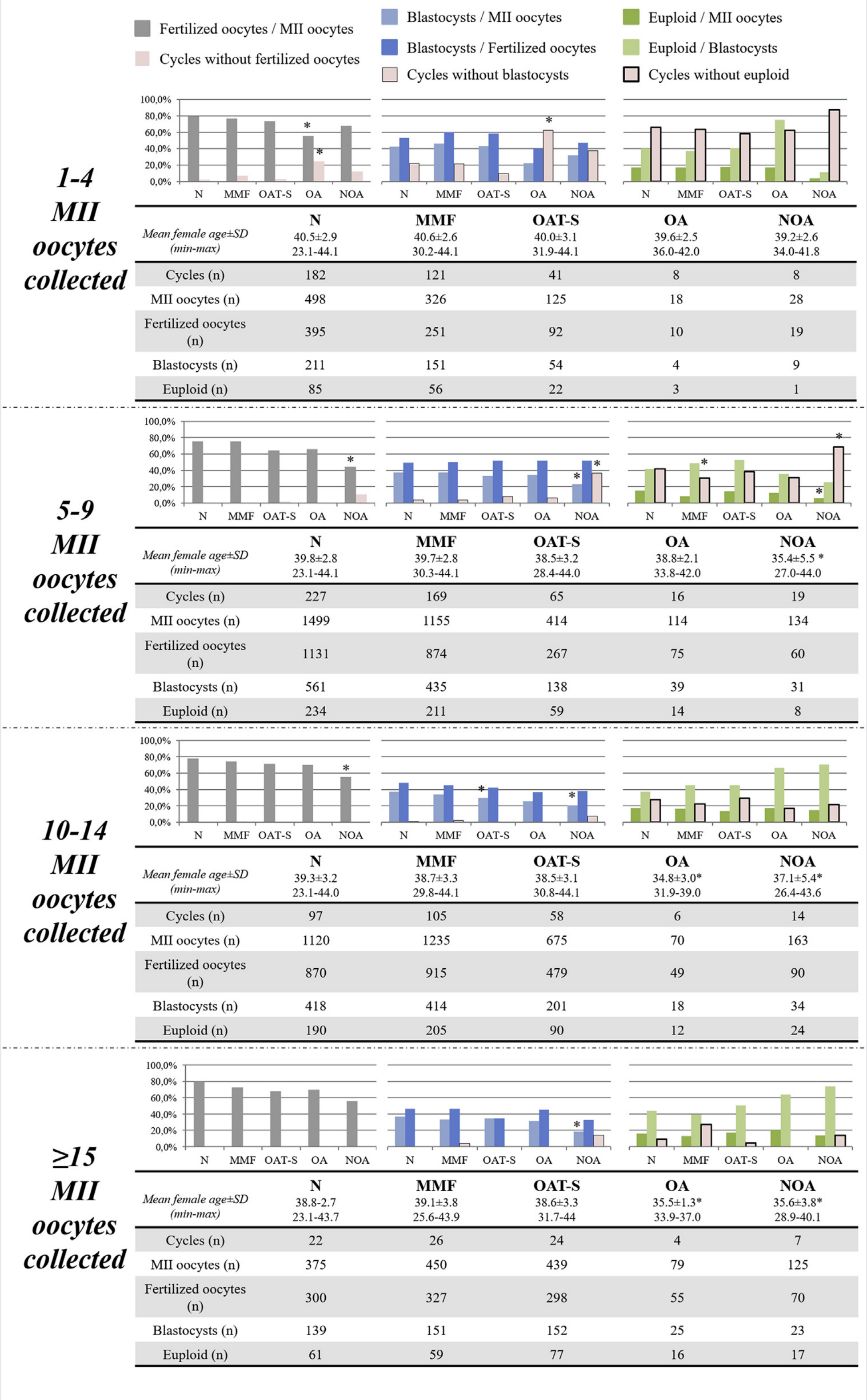
Female age ≥42 yr

	N	**MMF**	**OAT-S**	**OA**	**NOA**
Mean no. of MII±SD (min-max)	5.6±3.7 1-19	6.3±4.2 1-19	8.2±6.2* 1-26	3.6±3.6 1-8	8.9±2.9* 5-14
Cycles (n)	142	102	37	5	9
MII oocytes (n)	799	637	303	18	80
Fertilized oocytes (n)	599	463	201	14	44
Blastocysts (n)	225	189	86	7	21
Euploid (n)	41	42	16	0	6

Subanalysis of the embryologic and clinical results by female age at oocyte pick-up. *$P<.05$ versus N. MII = metaphase II; MMF = moderate male factor; N = normozoospermic; OA = obstructive azoospermia; OAT-S = sever oligoasthenoterazoospermia; NOA = nonobstructive azoospermia.

Mazzilli. Male factor and outcomes in PGT-A cycles. Fertil Steril 2017.

FIGURE 2



1-4 MII oocytes collected

Mean female age±SD (min-max)	N	MMF	OAT-S	OA	NOA
	40.5±2.9 23.1-44.1	40.6±2.6 30.2-44.1	40.0±3.1 31.9-44.1	39.6±2.5 36.0-42.0	39.2±2.6 34.0-41.8
Cycles (n)	182	121	41	8	8
MII oocytes (n)	498	326	125	18	28
Fertilized oocytes (n)	395	251	92	10	19
Blastocysts (n)	211	151	54	4	9
Euploid (n)	85	56	22	3	1

5-9 MII oocytes collected

Mean female age±SD (min-max)	N	MMF	OAT-S	OA	NOA
	39.8±2.8 23.1-44.1	39.7±2.8 30.3-44.1	38.5±3.2 28.4-44.0	38.8±2.1 33.8-42.0	35.4±5.5 * 27.0-44.0
Cycles (n)	227	169	65	16	19
MII oocytes (n)	1499	1155	414	114	134
Fertilized oocytes (n)	1131	874	267	75	60
Blastocysts (n)	561	435	138	39	31
Euploid (n)	234	211	59	14	8

10-14 MII oocytes collected

Mean female age±SD (min-max)	N	MMF	OAT-S	OA	NOA
	39.3±3.2 23.1-44.0	38.7±3.3 29.8-44.1	38.5±3.1 30.8-44.1	34.8±3.0* 31.9-39.0	37.1±5.4* 26.4-43.6
Cycles (n)	97	105	58	6	14
MII oocytes (n)	1120	1235	675	70	163
Fertilized oocytes (n)	870	915	479	49	90
Blastocysts (n)	418	414	201	18	34
Euploid (n)	190	205	90	12	24

≥15 MII oocytes collected

Mean female age±SD (min-max)	N	MMF	OAT-S	OA	NOA
	38.8-2.7 23.1-43.7	39.1±3.8 25.6-43.9	38.6±3.3 31.7-44	35.5±1.3* 33.9-37.0	35.6±3.8* 28.9-40.1
Cycles (n)	22	26	24	4	7
MII oocytes (n)	375	450	439	79	125
Fertilized oocytes (n)	300	327	298	55	70
Blastocysts (n)	139	151	152	25	23
Euploid (n)	61	59	77	16	17

Subanalysis of the embryological and clinical results by number of MII oocytes collected. *$P<.05$ versus N. Abbreviations as in Figure 1.

Mazzilli. Male factor and outcomes in PGT-A cycles. Fertil Steril 2017.

TABLE 2

Clinical outcomes by male factor.

Outcome	N	MMF	OAT-S	OA	NOA	Total
Total OPU, n	528	420	188	34	49	1,219
ET, n	361	339	151	27	23	901
Single ET, n (%)	357/361 (98.9%)	337/339 (99.4%)	145/151 (96.0%)	27/27 (100%)	22/23 (95.7%)	888/901 (98.6%)
Double ET, n (%)	4/361 (1.1%)	2/339 (0.6%)	6/151 (4.0%)	0/27	1/23 (4.3%)	13/901 (1.4%)
Positive pregnancy tests/ET, n, % (95% CI)	192/361 53.2% (48.0–58.3)	172/339 50.7% (45.4–56.0)	82/151 54.3% (46.4–62.1)	10/27 37.0% (21.5–55.8)	8/23 34.8% (18.7–55.2)	464/901 51.5% (48.2–54.8)
Positive pregnancy tests/OPU, n, % (95% CI)	192/528 36.4% (32.4–40.6)	172/420 41.0% (36.4–45.7)	82/188 43.6% (36.7–50.8)	10/34 29.4% (16.7–46.3)	8/49 16.3% (8.3–29.3)[a]	464/1,219 38.1% (35.4–40.8)
Biochemical pregnancy losses/positive pregnancy tests, n, % (95% CI)	18/192 9.4% (5.9–14.4)	12/172 7.0% (3.9–11.9)	6/82 7.3% (3.1–15.6)	1/10 10.0% (0–42.6)	1/8 12.5% (0–49.2)	38/464 8.2% (6.0–11.1)
Biochemical pregnancy losses/OPU, n, % (95% CI)	18/528 3.4% (2.1–5.4)	12/420 2.9% (1.6–5.0)	6/188 3.2% (1.3–6.9)	1/34 2.9% (0–16.2)	1/49 2.0% (0–11.7)	38/1,219 3.1% (2.3–4.3)
Miscarriages/clinical pregnancies, n, % (95% CI)	20/174 11.5% (7.5–17.2)	20/160 12.5% (8.2–18.6)	9/76 11.8% (6.1–21.2)	–	1/7 14.3% (0–53.4)	50/426 11.7% (8.8–15.2)
Miscarriages/OPU, n, % (95% CI)	20/528 3.8% (2.3–5.8)	20/420 4.4% (3.1–7.3)	9/188 4.8% (2.4–9.0)	–	1/49 2.0% (0–11.7)	50/1,219 4.1% (3.1–5.4)
Babies/ET, n, % (95% CI)	154/361 42.7% (37.8–47.8)	140/339 41.3% (36.2–46.7)	67/151 44.4% (36.7–52.3)	9/27 33.3% (18.5–52.3)	6/23 26.1% (12.3–46.8)	376/901 41.7% (38.5–45.0)
Babies/OPU, n, % (95% CI)	154/528 29.2% (25.4–33.2)	140/420 33.3% (29.0–38.0)	67/188 35.6% (29.1–42.7)	9/34 26.5% (14.4–43.3)	6/49 12.2% (5.4–24.6)[a]	376/1,219 30.8% (28.2–33.5)
Cycles with no pregnancy achieved and ≥1 euploid blastocyst yet to be transferred, n	31	33	20	1	4	90
Completed cycles, n (%)	497/528 (94.1%)	387/420 (92.1%)	168/188 (89.4%)	33/34 (97.1%)	45/49 (91.8%)	1,130/1,219 (92.7%)
Live birth rate in completed cycles, n, % (95% CI)	154/497 31.0% (26.9–35.3)	140/387 36.2% (31.4–41.2)	67/168 39.9% (32.8–47.4)	9/33 27.3% (14.9–44.4)	6/45 13.3% (50.5–26.8)[a]	376/1,130 33.3% (30.6–36.1)

Note: ET = embryo transfer; other abbreviations as in Table 1.

[a] $P < .05$.

Mazzilli. Male factor and outcomes in PGT-A cycles. Fertil Steril 2017.

that was generally higher than for N but reached statistical significance only for women <35 years of age (54.5% vs. 11.3%; $P<.05$; Fig. 1). The patients in the SMF groups collected a generally higher number of MII oocytes at OPU, but the lower fertilization and blastocyst rates balanced the results among the five study groups.

Figure 2 displays the results in the five study groups according to ranges of MII oocytes collected (1–4, 5–9, 10–14, and ≥15). The trends here reported generally mirrored the overall results. Specifically, the reduction in the fertilization and blastocyst rates per MII oocyte were common to all of the subanalyses conducted. However, this translated to a higher rate of cycles in which no euploid blastocyst was produced only for NOA versus N in the ranges 1–4 and 5–9 (statistical significance was reached solely in the latter subanalysis: 68.4% vs. 41.9%, respectively; $P<.05$; Fig. 2). The women in the SMF groups were generally younger than in N, but if the cycles overcame the impact of the male factor on fertilization and blastulation rates (especially when fewer than ten MII oocytes were collected at OPU), and at least one blastocyst was produced, the results per cycle were balanced among the five study groups owing to a higher (even if not statistically significant) euploidy rate per blastocyst obtained.

Clinical Outcomes

The number of ETs performed was 901: 361, 339, 151, 27, and 23 in N, MMF, OAT-S, OA and NOA, respectively. Overall, 98.6% of the ETs performed were single ET (n = 888/901), and the rates were similar in the five study groups (Table 2). The positive pregnancy test rates per ET were 53.2%, 50.7%, 54.3%, 37.0%, and 34.8% in N, MMF, OAT-S, OA, and NOA, respectively. The respective positive pregnancy test rates per OPU were 36.4%, 41.0%, 43.6%, 29.4%, and 16.3%, and the only statistically significant difference was reported in NOA compared with N ($P<.01$; Table 2). No statistically significant differences were reported in biochemical pregnancy loss and miscarriage rates (Table 2). At the time of writing, the total number of babies born was 376 (1,130 out of 1,219 started cycles [92.7%], were complete due to either a full-term delivery or the completion of all possible ETs without the achievement of a pregnancy). The live birth rate per ET was not statistically different among the study groups (42.7%, 41.3%, 44.4%, 33.3%, and 26.1%). However, the live birth rate per OPU was significantly reduced in NOA compared with N (12.2% vs. 29.2%; $P=.01$; Table 2).

Perinatal and Obstetrical Outcomes

No difference was reported among N, MMF, and SMF (OAT-S, OA, and NOA) regarding birth weight, gestational age (Supplemental Fig. 3), and congenital malformations. In only eight (2.1%) of the 376 babies born was a congenital malformation reported (four atrial septal defects, two patent foramen ovale, one esophageal atresia, and one pes tortus).

Logistic Regression Analyses and Post Hoc Power Calculation

Two logistic regression analyses were conducted to investigate all of the possible basal and cycle confounders on the likelihood of a couple to obtain a transferable blastocyst per oocyte retrieval and on the likelihood of each single blastocyst to have a euploid constitution (Supplemental Table 2). Only female age (odds ratio [OR] 0.75, 95% confidence interval [CI] 0.71–0.8; and 0.79, 95% CI: 0.77–0.81; $P<.001$) was shown as a significant predictor in both analyses (Supplemental Table 2). In contrast, the number of MII oocytes collected (OR 1.19, 95% CI 1.15–1.24), and NOA sperm parameter (OR 0.5, 95% CI 0.25–0.99; $P=.05$) were significantly correlated with the outcome only from a per-cycle–based perspective ($P<.01$; Supplemental Table 2), but not from a per-blastocyst–based one. Finally, the blastocyst morphologic quality also was shown to be correlated with the likelihood of each single blastocyst to be euploid ($P<.01$; Supplemental Table 2).

The post hoc power calculation (two-sided level of significance = .05) highlighted a 0.99 statistical power to rule out a 2% difference when the euploidy rate per inseminated oocyte was 16.3%.

DISCUSSION

To date, little is known about the impact of the male factor on IVF outcomes such as embryo developmental competence and, more importantly, on aneuploidy rate.

In this study 1,219 cycles conducted for 1,090 couples were included, and the ICSI outcomes were evaluated according to seminal characteristics and male partner personal features. For NOA, 51.5% of the cycles started were excluded because spermatozoa were not recovered after TESE. These data are consistent with other studies: Vloeberghs et al. (2) reported a successful sperm retrieval in 40.5% of NOA, Cissen et al. 43.7% (48), and Verheyen et al. 50% (49).

The results of this study show that in the early stages of in vitro embryonic development there is a difference between azoospermic/OAT-S and normozoospermic subjects regarding oocyte fertilization and blastocyst development. Significantly lower fertilization rates were observed when testicular spermatozoa from men with NOA were used for ICSI. Impaired fertility potential when testicular sperm are used have been found by other groups also (50–53). Testicular spermatozoa are less mature and, therefore, less competent than ejaculated sperm, where further maturation occurs in the epididymis. This could explain the higher fertilization failure and lower developmental rates to blastocyst (52). SMF (OA, NOA, and OAT-S) in the present study was not determinant of the morphologic and genetic quality (euploidy) of the blastocysts produced. This could be either explained by the oocytes' potential to prevent the further development of aneuploid embryos before the embryonic genome activation (54), or because sperm-derived aneuploidies may result in an early interruption of embryo development (53). Our results partially disagree with some previous reports. Specifically, Magli et al. (40) showed that SMF may contribute to a higher prevalence of aneuploid blastocysts (55% aneuploidy rate with N, 62% with oligozoospermia, and 69% with NOA), but that analysis was performed on day-3 embryos and with the use of 9-chromosome FISH technique. Silber et al. (41) compared the prevalence of aneuploidies and mosaicism in ICSI cycles performed in patients with oligozoospermia and azoospermia with sperm retrieved by means of TESE: The embryos were euploid in 41% of the patients with OAT compared with 22% in those with azoospermia, but again the analysis was conducted with the use of 9-chromosome FISH. Coates et al. (55) instead performed aCGH on trophectoderm biopsies and reported that SMF was associated with a significant increase of sex chromosome abnormalities in blastocysts compared with subjects with normozoospermia.

At the time of writing, 92.7% of the started cycles in the present study were complete. The live birth rate per OPU was similar among the study groups, except for NOA compared with N. These data confirm the previously described impaired fertility potential in NOA subjects. In accordance with a recent report (56), no statistically significant differences were found in our study regarding biochemical pregnancy loss and miscarriage rates. Furthermore, regarding perinatal and obstetrical outcomes, no differences were reported among the groups in gestational age, birth weight, and congenital malformations. Thus, in the present study, SMF seemed not to have any negative effect on fetal postimplantation development, even if a higher number of babies born are required to confirm these preliminary data. These results support what has been previously described by Tsai et al. (57), but they are in disagreement with Fedder et al., who reported a higher rate of cardiac malformations and hypospadias after ICSI with the use of epididymal or testicular sperm (58). Both of those studies, however, were biased by a multiple pregnancy rate ranging from 20% to 40%, which may per se affect the obstetrical and perinatal outcomes (59), although our data derive only from singleton pregnancies after elective single euploid blastocyst transfer. The overall prevalence of congenital malformations (2.1%) here assessed was similar to what has been previously reported after either IVF with and without PGT-A (60, 61) or spontaneous conceptions (62–64). In general, though, more data are required from future studies to draw a clear conclusion on these still controversial issues.

Obviously, ICSI outcomes may be affected by confounding factors, such as female age, history and duration of infertility, miscarriage, and lifestyle (BMI, smoking, etc.), which should be carefully evaluated (27, 28, 30–36, 65–67). In the present study, to limit the influence of a better prognosis from the maternal perspective in the SMF groups (younger women and higher number of MII oocytes collected) on the outcomes, the results were also reported by ranges of female age (<35, 36–38, 39–41, and ≥42 years) at OPU and by ranges of MII oocytes collected (1–4, 5–9, 10–14, and ≥15), which highlighted trends similar to the overall embryologic and clinical analyses. In general, azoospermic couples tend to be characterized by younger female partners with a better ovarian reserve and response to the stimulation, because they acquire an indication for IVF-ICSI earlier. They also tend to undergo a higher number of attempts compared with N, owing to a generally higher number of cycles in which no zygote or blastocyst is produced, particularly when fewer than ten MII oocytes are collected at OPU. When at least one blastocyst was obtained, however, the euploidy rate was not affected by the male factor in any of the subanalyses conducted.

Previous data dealing with the investigation of a putative impact of male age on ICSI outcomes are discordant. Some authors attempted to define a cutoff for “advanced paternal age”: ≥35 (20–23, 68), ≥40 (24, 25), and ≥50 (26) years have been suggested. Some reports (21–23, 68) showed that advanced paternal age is associated with an increased prevalence of sperm DNA fragmentation and damage, and it could impair IVF outcomes, though not those after ICSI (39). Meijerink et al. conducted a retrospective study of 7,051 ICSI cycles and showed no statistically significant effect of paternal age on embryo quality and biochemical and clinical pregnancy rates (20). In our study, the logistic regression analyses regarding the likelihood of a couple to obtain a transferable blastocyst per oocyte retrieval and the likelihood of each single blastocyst to be euploid showed that male age did not have any correlation, in contrast to what is reported for female age.

The same analysis revealed that the sperm factor can be considered to be a significant predictor only from a per-cycle–based perspective for NOA compared with N, and not from a per-blastocyst–based one. Thus, these results highlighted that impaired spermatogenesis may have a negative effect on only the early stages of embryo development.

Likewise, also male smoking and BMI were irrelevant, in accordance with some studies (27, 35–38, 48, 69–74) and in disagreement with others (28–30, 32, 34). Specifically, Bakos et al. (30) reported a pregnancy rate of 47.3% in normal-weight men and 23.4% in high-BMI men. Other authors (33, 35, 36) showed no correlation between BMI and outcome of ICSI.

SMF, especially NOA, is negatively related to fertilization and embryo developmental potential. For this reason, it is strongly suggested to improve spermatogenesis before starting ICSI, if possible, for example, with the use of FSH therapy in case of hypogonadotropic hypogonadism, antibiotics and antiinflammatory drugs in case of infections, or aromatase inhibitors (75, 76).

In general, but especially for AMA patients, it could be important to increase the number of MII oocytes collected to maximize the number of blastocysts produced. In fact, once a blastocyst is obtained, the male factor does not seem to affect the aneuploidy rate, eventually the implantation rate, and possibly the postimplantation development of euploid embryos. Double stimulation in the same menstrual cycle (Duostim) (77, 78) or oocyte accumulation policy (79) might be possible clinical strategies in poor-prognosis women.

The present study is limited by its retrospective fashion and the sample size. In particular, a higher number of azoospermic subjects is desirable for future prospective studies, especially to provide more solid evidence investigating the clinical outcomes (pregnancy and obstetrical and perinatal results), for which the present study was underpowered.

In conclusion, SMF impairs early embryonic competence regarding fertilization rate and developmental potential. However, the euploidy rate and implantation potential of the obtained blastocysts are independent from sperm quality. If the results shown here are confirmed in other studies and from other IVF centers, SMF should be disregarded in the future as an indication for PGT-A.

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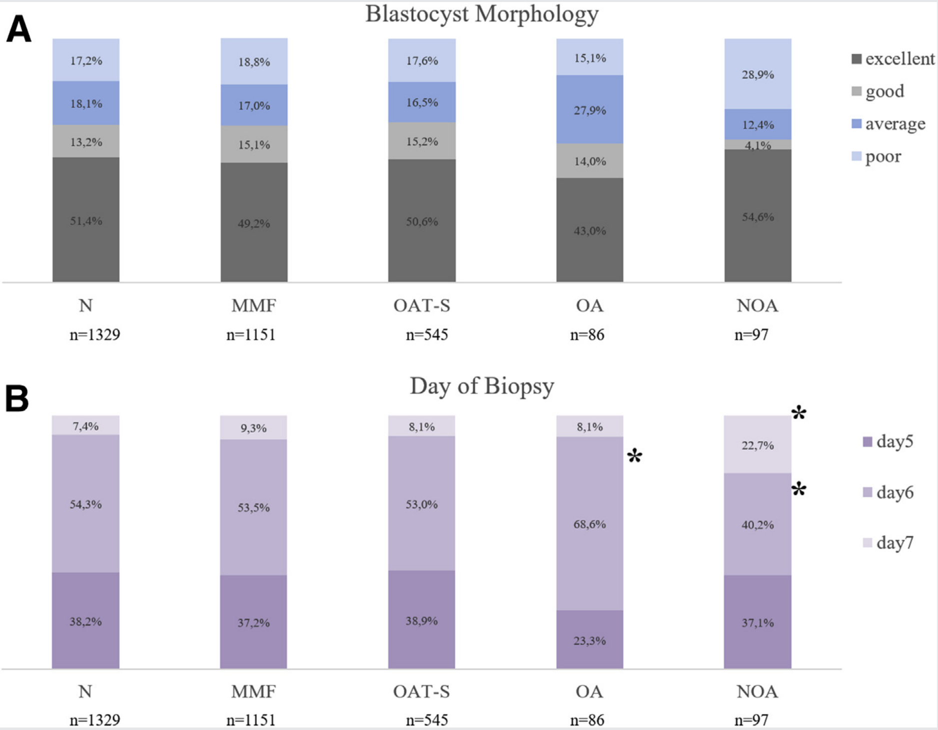
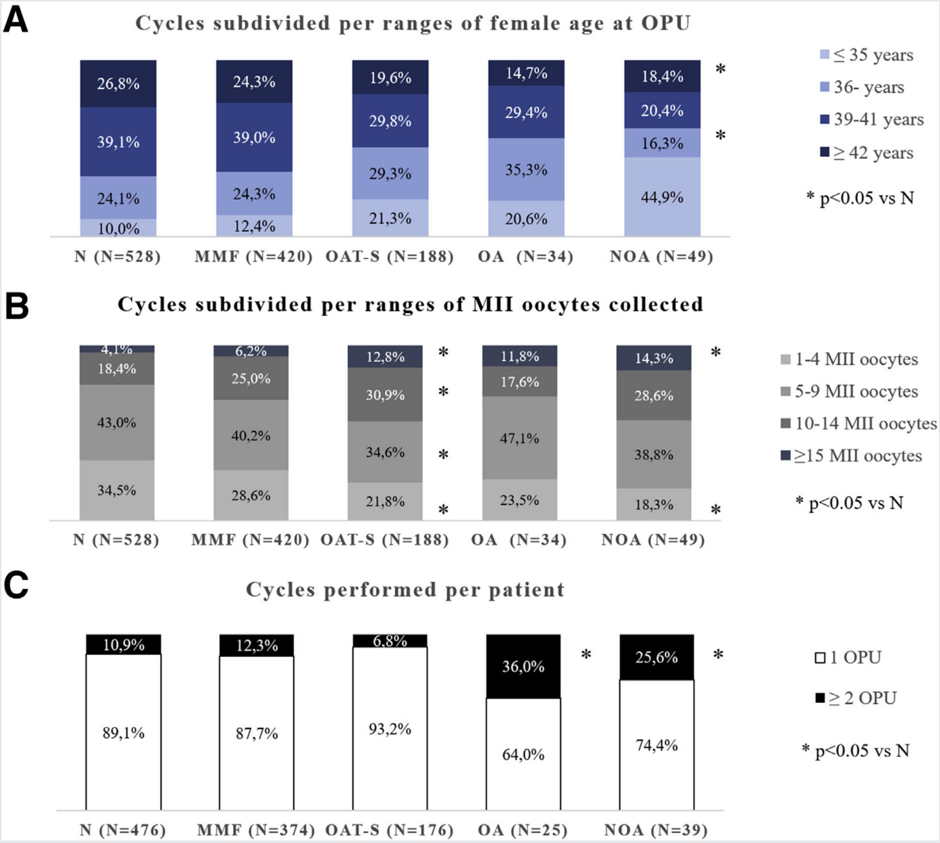
SUPPLEMENTAL FIGURE 1



(**A**) Blastocyst morphology and (**B**) day of biopsy by sperm factor. *$P<.05$ versus N. MII = metaphase II; MMF = moderate male factor; N = normozoospermic; OA = obstructive azoospermia; OAT-S = severe oligoasthenoterazoospermia; NOA = nonobstructive azoospermia.

Mazzilli. Male factor and outcomes in PGT-A cycles. Fertil Steril 2017.

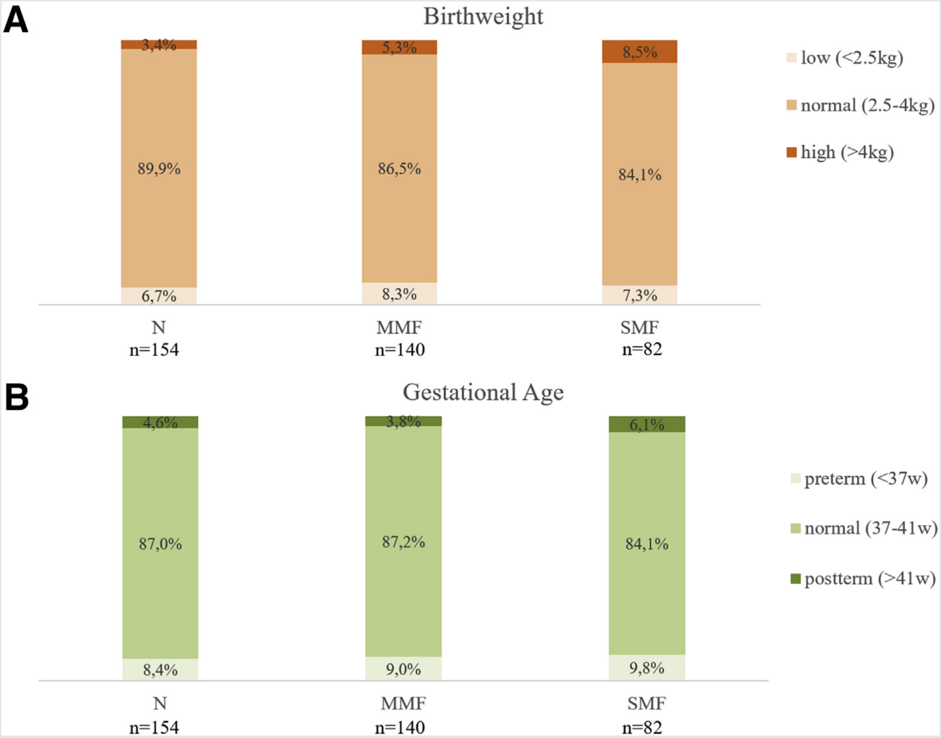
SUPPLEMENTAL FIGURE 2



Distribution of the cycles by (**A**) female age at oocyte pick-up (OPU), (**B**) number of MII oocytes collected, and (**C**) patient. *$P<.05$ versus N. Abbreviations as in Supplemental Figure 1.

Mazzilli. Male factor and outcomes in PGT-A cycles. Fertil Steril 2017.

SUPPLEMENTAL FIGURE 3



(**A**) Birth weight and (**B**) gestational age by sperm factor. SMF = severe male factor (which includes OAT-S, OA, and NOA). Other abbreviations as in Supplemental Figure 1.

Mazzilli. Male factor and outcomes in PGT-A cycles. Fertil Steril 2017.