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Can sperm quality influence embryo development and its ploidy? Analysis of 811 blastocysts obtained from different sperm sources

Romualdo Polese[1], Filomena Scarselli[2], Brian Dale[1], Maria Giulia Minasi[2] and Ermanno Greco[2]

[1]Centro Fecondazione Assistita. Napoli, Campania, Italy and [2]European Hospital, Centre for Reproductive Medicine. Rome, Italy

Research Article

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Author for correspondence:
Romualdo Polese. Centro Fecondazione Assistita. Napoli, Campania, Italy.
E-mail: polese.romualdo@gmail.com

Summary

The aim of our study was to evaluate the correlation between sperm quality and ploidy status of the derived blastocysts. We performed a retrospective analysis on a restricted pool of patients enrolling only those who had no female factors. Male patients with genetic factors affecting spermatogenesis were also excluded. We chose a maternal age ≤38 years to decrease the female factor, therefore the male factor was the main component of sterility. We divided the patients in four groups based on semen quality and comparing fertilization, pregnancy and euploidy rates above all. In total, 201 intracytoplasmic sperm injection (ICSI) cycles were enrolled in the study. Cycles were divided into four groups, according to semen source: normal semen, oligoasthenoteratozoospermia (OAT), cryptospermia or non-obstructive azoospermia (NOA). An extremely statistically lower fertilization rate was found in NOA patients. Unexpectedly, no differences were detected in blastocyst formation, euploidy, aneuploidy and mosaicism rates among the four groups. Interestingly, we also found a higher abortion rate comparing NOA to normal semen with an odds ratio of 4.67. In our study no statistically significant differences among the analyzed groups were found, showing little or no effect at all using spermatozoa from different semen sources or quality. This may be linked to the oocyte competence of fixing sperm DNA damage and it could be hypothesized that only sperm with a good rate of DNA integrity are able to fertilize the oocyte, explaining why poor quality semen is reflected in a low fertilization rate without effect on ploidy.

Introduction

Infertility affects approximately 15% of couples with 50% of cases being attributed to a male factor (Chandra *et al.*, 2005). The main causes of altered male factor fertility are many: oligozoospermia, asthenozoospermia, teratozoospermia, cryptozoospermia and azoospermia. In the ICSI procedure the physiological mechanisms of natural sperm selection are bypassed and the sperm injected into the oocyte is selected directly by the biologist, combining attention to choose with motility and normal gross morphology. This procedure does not ensure the genetic integrity of the chosen sperm and, consequently, of the derived embryo. Moreover, to date, the consequences of using low quality spermatozoa are still unclear. Some studies have disclosed that offspring conceived by ICSI have been shown to be at increased risk for aneuploidies, with a higher incidence on sex chromosomes (Hansen *et al.*, 2005; Ludwig, 2005). To date, the consequences of using low quality sperm is still under discussion. Some authors report a direct correlation between extensive teratozoospermia and impaired implantation and ongoing pregnancy rates (Taşdemir *et al.*, 1997); this might be related to higher rates of DNA fragmentation, mitochondrial dysfunction and chromosomal aneuploidy in sperm of oligozoospermic and/or asthenozoospermic and/or teratozoospermic men compared with unaffected controls (Jiang *et al.*, 2015). Sperm DNA testing has been increasingly used as an additional parameter of sperm evaluation (Bungum *et al.*, 2004; Virro *et al.*, 2004; Check *et al.*, 2005; Benchaib *et al.*, 2007; Bungum *et al.*, 2007; Frydman *et al.*, 2008; Ozmen *et al.*, 2007; Lin *et al.*, 2008). Sperm DNA quality might be one of the important determinants of normal fertilization and embryo development. For this reason, many studies have investigated the relationship between outcome of assisted reproductive technology (ART) and high DNA damage in sperm (Aitken *et al.*, 1998; Lopes *et al.*, 1998; Henkel *et al.*, 2003; Larson-Cook *et al.*, 2003; Gandini *et al.*, 2004; Dar *et al.*, 2013; Simon *et al.*, 2013). However, most studies have found that sperm with DNA damage were capable of fertilizing an oocyte (Zini *et al.*, 2005; Borini *et al.*, 2006), showing only a modest effect on conception rates with conventional IVF and almost no effect with ICSI (Aitken *et al.*, 1998; Zini *et al.*, 2005; Borini *et al.*, 2006; Bungum *et al.*, 2007; Kobayashi *et al.*, 2007; Ozmen *et al.*,

2007; Collins *et al.*, 2008; Frydman *et al.*, 2008; Lin *et al.*, 2008; Zini *et al.*, 2008; Dar *et al.*, 2013; Simon *et al.*, 2013). Conversely, Zini *et al.* (2008) reviewed these observations concluding that sperm DNA damage was associated with a significantly higher risk of pregnancy loss after IVF and ICSI. Furthermore, aberrant DNA methylation of imprinted loci in sperm from oligospermic patients has been reported (Kobayashi *et al.*, 2007). This result might be related to a low degree of maturity and a high malformation rate in testicular sperm (Lu *et al.*, 2012). A recent study outlined that the use of suboptimal sperm increases the risk of aneuploidy of sex chromosomes in preimplantation blastocyst embryos (Coates *et al.*, 2015). Many studies have focused on the risk of using frozen sperm as ICSI allows the use of thawed sperm even if cryopreservation impairs semen motility; some studies have shown that using thawed sperm could be not as safe as it seems, as the accumulated evidence shows that the process of freezing and thawing could cause damage to the sperm (Giraud *et al.*, 2000; Chatterjee and Gagnon, 2001).

Probably the most addressed issue is the use of testicular or epididymal sperm that is considered to have a low degree of maturity and therefore leading to a high malformation rate (Takahashi, 2012). Tsai *et al.* (2015) compared clinical outcome of obstructive azoospermia (OA) and non-obstructive azoospermia (NOA) couples after testicular sperm extraction (TESE) and ICSI, and found that the two groups had similar clinical pregnancy, chemical pregnancy, implantation, live birth and abortion rates per transfer. They also compared perinatal outcomes and the subsequent development of the children over a median of 10 years following TESE–ICSI, detecting no statistical significant difference in live births rates. Moreover, the health of the children conceived using ICSI was satisfactory, without any major retardation in psychomotor or intellectual development (Ludwig *et al.*, 2009). The development of children conceived by ICSI with extracted testicular sperm or with ejaculated sperm from men with extreme severe OAT was found to be comparable (Tsai *et al.*, 2011, 2015). In light of the above-mentioned findings, it is mandatory to understand if the use of sperm retrieved from different sources or the use of sperm that is clearly abnormal may influence not only the fertilization rate but also embryo development or embryo ploidy, increasing the risk of contracting a genetic disease. The aim of our study was to analyze possible relationships between sperm quality and chromosomal defect through blastocyst preimplantation genetic testing (PGT) analysis.

Materials and methods

Subjects

Our study is a retrospective analysis of 201 ICSI-PGT cycles performed from January 2012 to December 2015 to evaluate the incidence of chromosomal aneuploidy in patients who submitted to the following selection criteria: maternal age ≤38 years old, no intracytoplasmic morphologically selected sperm injection (IMSI), no genetic diseases. Couples with female infertility factors such as endometriosis, low ovarian reserve, polycystic ovary syndrome (PCOS) and genetic indications were excluded from the study. Only male patients with a normal karyotype were enrolled in the study. We chose a maternal age ≤38 years old to decrease the female factor. Mean female age, anti-Müllerian hormone (AMH) values, body mass index (BMI) and E_2 values highlighted no statistical significant difference between the four groups; the same stand true for male mean age.

Group 1 was composed of 95 cycles with normal semen parameters based on WHO (2010) guidelines (total sperm number, calculated by sperm concentration × sperm volume, ≥39 × 10^6 sperm/ml, progressive motility ≥32%, and morphology ≥4% normal forms). Group 2 was composed of 58 cycles with oligoasthenoteratozoospermia (OAT) (total sperm number <39 × 10^6, progressive motility <32% morphology <4%). Group 3 was composed of 29 cycles including extremely severe OAT, i.e. cryptospermic men (sperm concentration from 0.0003 to 1 × 10^6/ml). Group 4 was composed of 19 cycles including NOA.

Sperm preparation

Fresh ejaculated semen was collected on the day of oocyte retrieval and left to liquefy for ~20–30 min prior to semen analysis. Afterwards, the semen specimens were processed using the swim up procedure; in this kind of selection the sperm was washed using 5 ml of sperm washing medium and centrifuged for 10 min at 600 *g*; therefore, the supernatant was carefully removed leaving only the pellet that was suspended by slowing agitating the tube. Finally, 500 µl of cleavage medium with HEPES, 5% human serum albumin (Quinn Sage, CooperSurgical, Pasadena, USA) was stratified leaving the tubes for 30 min at 37°C in 6% CO_2 and then 200 µl were taken and transferred into a new tube and put in the incubator. The surgical procedure was performed under general anaesthesia, as described elsewhere (Franco *et al.*, 2016). Briefly, after disinfection the scrotum was incised along the scrotal raphe and the testis was then delivered through the incision. Sperm extraction was always performed using multiple biopsies (4–8 samples). Tissue samples were placed in a Petri dish (containing 6–8 ml of buffered medium with HSA) in which the seminiferous tubules were opened by mechanically dissection with glass coverslides (wet preparation). The sperm search on the wet preparation was performed at ×400 magnification under an inverted microscope. After ~10–15 min, whether or not sperm are found in the wet preparation, the TESE sample was centrifuged (600 *g* for 10 min) in 0.5 ml of a buffered medium (Quinn's Advantages Medium with HEPES, 5% human serum albumin; Sage, CooperSurgical, Pasadena, USA). Part of the cell suspension was smeared and covered with mineral oil in order that sperm search could continue on a more concentrated sample.

Ovarian stimulation, oocyte denudation and insemination

Controlled ovarian stimulation was performed using recombinant follicle stimulating hormone (FSH) (Gonal F, Merck Serono, Geneva, Switzerland) and gonadotropin-releasing hormone (GnRH)-antagonist flexible protocol. Evaluation of the patient's age, BMI, antral follicle count. (AFC) and AMH were taken into account in the recombinant FSH starting dose calculation. The number and the mean diameter of the growing follicles were assessed by means of periodic transvaginal ultrasound scans. Together with serum estradiol levels, these data were used to adjust the recombinant FSH dose. When at least three follicles reached 19 mm in diameter, human chorionic gonadotrophin (hCG) (Gonasi, 10,000 IU, IBSA, Lodi, Italy) was administered by intramuscular injection. Oocyte pick-ups were performed 36–38 h later by ultrasound-guided transvaginal follicular puncture. The ovarian stimulation method leading to supernumerary oocyte retrieval has been described in detail elsewhere (Litwicka *et al.*, 2018). Briefly, controlled ovarian stimulation was performed using recombinant FSH (Gonal F, Merck Serono, London, UK) and gonadotropin-releasing hormone agonist in a long suppression protocol or GnRH-antagonist

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protocol according to ovarian reserve and AMH values. When at least three follicles reached 18 mm in diameter, hCG (Gonasi, 10,000 IU, IBSA, Lodi, Italy) was administered by intramuscular injection. Oocytes were retrieved 36–38 h later by ultrasound-guided transvaginal follicular puncture.

The oocytes were stripped of the surrounding cumulus cells (38 h after hCG administration) by brief exposure to 20 IU/ml hyaluronidase (Quinn, Sage hyaluronidase, CooperSurgical, Pasadena, USA) in buffered medium (Quinn's Advantages Medium with HEPES, 5% human serum albumin, Sage, CooperSurgical, Pasadena, USA) under oil. Subsequently, oocytes were gently aspirated in and out of a plastic pipette (Flexipet, 170 and 140 µm i.d.; Cook, Australia) to allow the complete removal of cumulus and corona cells. The denuded oocytes were then observed and evaluated. Mature oocytes were therefore moved into an ICSI dish ready for the procedure.

The ICSI procedure was performed 38–42 h post-hCG injection. The microinjection procedure was performed on a heated stage at 37°C under an inverted microscope at ×400 magnification. Sperm were selected at the periphery of the polyvinylpyrrolidone (PVP; Sage, CooperSurgical, Pasadena, USA) microdroplet. A selected spermatozoon was immobilized by repeatedly touching the tail and was then aspirated, tail first, into the injecting pipette. The oocyte was held by a holding pipette with the polar body at the 6 o'clock position. The injecting pipette containing the spermatozoan was introduced at the 3 o'clock position through the zona pellucida and oolemma, deep into the cytoplasm. A small part of the cytoplasm was aspirated. The spermatozoon and the cytoplasm were injected, and the injecting pipette was withdrawn gently. After injection, the oocytes were rinsed and incubated in cleavage medium with HEPES, 5% human serum albumin (Quinn, Sage, CooperSurgical, Pasadena, USA) at 37°C, in 6% CO_2, 5% O_2.

Embryo culture

Fertilization was confirmed 16–20 h post-ICSI procedure by the presence of two pronuclei. Embryo culture was realized using Sanyo incubators (model MCO-5M) at 37°C in 6% CO_2 5% O_2. Embryos were grown in cleavage medium supplemented with HEPES, 5% human serum albumin (Quinn, Sage, CooperSurgical, Pasadena, USA) until day 3 when they were switched to blastocyst medium supplemented with HEPES, 5% human serum albumin (Quinn, Sage, CooperSurgical, Pasadena, USA) and laser assisted hatching was performed. When the blastocyst stage was reached, from day 5 to day 7 of culture, the trophectoderm (TE) biopsy was carried out.

Trophectoderm biopsy and genetic analysis

Trophectoderm biopsy was performed at the blastocyst stage, as described elsewhere (Minasi *et al.*, 2016). Briefly, on the day of biopsy, 5–10 TE cells were aspirated using a biopsy pipette, washed in sterile phosphate-buffered saline solution (PBS) and finally placed into microcentrifuge tubes containing 2 µl PBS and sent to the Genoma Laboratory for genetic analysis. For whole-genome amplification (WGA), TE cells were first lysed and genomic DNA was randomly fragmented and amplified using the SurePlex DNA Amplification System (BlueGnome, Cambridge, UK). Briefly, WGA products were fluorescently labelled and competitively hybridized to 24sure V3 arrays (BlueGnome) to a matched control in an aCGH experiment format. Therefore, a laser scanner InnoScanw 710 AL (INNOPSYS, Carbonne, France) was used to excite the hybridized fluorophores and read and store the resulting images of the hybridization. The images were analyzed and quantified by algorithm fixed settings in BlueFuse Multi Software (BlueGnome) (Gutiérrez-Mateo *et al.*, 2011).

Blastocyst vitrification and warming

All blastocysts were cryopreserved after biopsy. Vitrification and warming were carried out with the use of the Kuwayama protocol with a Cryotop as support (Kuwayama 2007). During vitrification, two solutions (Kitazato Vitrification Kit, BioPharma, Shizuoka, Japan) were used: equilibration (7.5% ethylene glycol and 7.5% dimethyl sulfoxide) and vitrification (15% ethylene glycol, 15% dimethyl sulfoxide, and 0.5 mol/l sucrose) solutions. Blastocysts were incubated at room temperature for 15 min in the first one and moved for another 30–60 s to the second one. Finally, blastocysts were individually loaded onto a Cryotop and quickly plunged into liquid nitrogen. During warming, three solutions (Kitazato Warming Kit, BioPharma, Shizuoka, Japan) were used: thawing (1 mol/l sucrose), dilution (0.5 mol/l sucrose) and washing (without sucrose) solutions. Blastocysts were incubated at 37°C for 1 min in the first solution and then moved at room temperature to the other two solutions for 3 and 5 min, respectively. Blastocysts were finally incubated at 37°C, in 6% CO_2, 5% O_2 for 2 h before transfer.

Blastocyst transfer

Single frozen–thawed embryo transfer (FET) was performed in patients prepared by combining gonadotropin-releasing hormone agonist and oestrogen pills (Progynova, Bayer, New Zealand Limited, Auckland). All transfer procedures were carried out with the use of a catheter (Wallace, Smits-Medical, Dublin, Ireland) under direct ultrasound guidance as previously described (Greco *et al.*, 2016). No blastocyst transfer was done with an endometrium thickness of <7 mm. Intramuscular administration of progesterone in oil (Prontogest, IBSA, Lodi, Italy) was initiated 6–7 days before embryo transfer and continued until the first serum β-hCG determination.

Ethical approval

All the procedures reported in this study are routinely applied in our infertility centre. For this reason, the Institutional Ethics Committee of the European Hospital approved the present study proposal in accordance with the Helsinki Declaration. Informed consent forms were signed from all the patients enrolled in this study.

Statistical analysis

Quantitative data are shown as mean ± standard deviation (SD). Categorical data are presented as numbers and percentages. Chi-squared test, or Fisher's exact test when necessary, was used to compare categorical data. Analysis was performed using STATA 14.2 (Stata; Data Analysis and Statistical Software, TX, USA); a P-value < 0.05 was considered statistically significant.

Results

In total, 201 ICSI cycles were taken into account. Group 1 included 95 cycles: 930 mature eggs were injected forming 731 embryos (78.6% fertilization rate); 399 embryos reached the blastocyst stage (54.6% blastocyst formation rate) of which 364 underwent

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trophectoderm biopsy: 161 blastocysts were euploid (44.2%), 165 were aneuploid (45.3%) and 36 were genetic mosaic (9.9%).

Group 2 included 58 cycles: 567 mature eggs were injected forming 384 embryos (67.7% fertilization rate); 217 embryos reached blastocyst stage (56.5% blastocyst formation rate) of which 209 underwent to trophectoderm biopsy: 93 blastocyst were euploid (43.7%), 91 were aneuploid (43.5%) and 22 were genetic mosaic (10.5%).

Group 3 included 29 cycles: 305 mature eggs were injected forming 213 embryos (69.8% fertilization rate); 125 embryos reached blastocyst stage (58.7% blastocyst formation rate) of which 122 underwent trophectoderm biopsy: 61 blastocyst were euploid (50.4%), 49 were aneuploid (40.5%) and 10 were genetic mosaic (8.3%).

Group 4 included 19 cycles: 231 mature eggs were injected forming 143 embryos (61.9% fertilization rate); 70 embryos reached blastocyst stage (49.0% blastocyst formation rate); all blastocysts underwent trophectoderm biopsy: 29 blastocyst were euploid (41.4%), 31 were aneuploid (44.3%) and 10 were genetic mosaic (14.3%) (Table 1).

A comparison between blastocyst formation, euploidy, aneuploidy and mosaicism in the four groups showed no statistically significant difference while, as expected, an extremely statistically significant difference was found comparing fertilization rates among groups, showing a statistically lower fertilization rate in NOA patients compared with the others (Table 1). Interestingly, we found a statistically higher abortion rate when comparing NOA to normal semen with an odds ratio = 4.67 (Table 2). We did not find statistical differences in all other analyzed parameters, as shown in Tables 1 and 2.

On the basis of the Coates *et al.* (2015) work, we analyzed each chromosomal incidence in monosomy, complex aneuploidy and mosaicism without finding any statistical significant pattern (Tables S1–S4).

Discussion

Embryo aneuploidy is the most common cause of embryo implantation failure and early pregnancy loss (Simon *et al.*, 2014), it is mainly attributed to oocyte chromosomal abnormalities that can cause modifications in the assembly of meiotic spindle, leading to errors in both chromosome alignment and microtubule matrix, increasing frequency of chromosomal degeneration into unassociated chromatids or increasing rates of chromosomal nondisjunction (Holubcová *et al.*, 2015). These abnormalities increase with maternal age, causing an increased risk of chromosomal abnormality in the produced embryos. At this time, embryo genetic screening by different molecular techniques (qPCR, aCGH, NGS) at blastocyst stage is currently used to detect chromosome aneuploidies and to select the embryo for transfer. This technique can be particularly indicative to disclose the effect of the different sperm pathologies on blastocyst aneuploidies when it is adopted in young women, who should have a reduced effect of aneuploidies related to aged oocytes. In our study, we analyzed the relationship between semen quality, embryo development and ploidy. According to our data, there was no evidence of a different embryo development after ICSI based on sperm quality. By enrolling in the study patients without any known female factors and with a maternal age ≤38 years old, we conceivably selected only male factor cycles. Probably limiting female age is one of the reasons that led to these results. Simon *et al.* (2013) showed that implantation rates are significantly higher if the female age is ≤35 years old compared with older women in ICSI cycles performed with a highly fragmented sperm DNA (Coates *et al.*, 2015).

In our study, we found a difference among the fertilization rates in the four groups, which is, as expected, significantly lower in the TESE group. Numerous studies, including the present work, supported the conclusion that poor sperm morphology on pre-IVF semen analysis using Kruger's strict criteria does not correlate to either poor fertilization and/or pregnancy rates in ICSI cycles (Küpker *et al.*, 1995; Nagy *et al.*, 1995; Svalander *et al.*, 1996; Lundin *et al.*, 1997; McKenzie *et al.*, 2004; Keegan *et al.*, 2007; MacLennan *et al.*, 2015).

An unexpected finding was the absence of variation in the blastulation rates among the study groups, although blastocysts derived from sperm obtained from TESE seemed to have a slower development (there was a higher ratio of day-6 blastocysts in Group 4 compared with the others). Subfertile patient populations are known to possess in their spermatozoa significantly elevated levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG), which is particularly mutagenic (De Iuliis *et al.*, 2009; Aitken *et al.*, 2010; Lord and Aitkin, 2015). ICSI has been reported to increase the probability of spermatozoon harbouring mutagenic lesions that will achieve fertilization (Chabory *et al.*, 2009). Conversely, zygotes can partially fix DNA damage (Lu *et al.*, 2012; Lane *et al.*, 2014). This might explain why in younger women treated in our study there was no statistically significant difference in euploidy rate of the blastocysts obtained using spermatozoa from the four different sources. Our data correlated with those published by Lu *et al.* (2012), who did not find any difference in pregnancy rates analyzing 1925 ICSI cycles independently from the semen origin. Anyway, they found a difference in the embryo morphology that was partially correlated with the slower development found in our study in the testicular group. They divided the patients in seven groups based on the sperm quality or sperm source, finding a lower fertilization rate in extreme OAT or obstructive azoospermia patients, although without detecting differences in pregnancy rates, even if the average number of embryo transferred in the groups with a lower quality semen was statistically lower. It is interesting to underline that the mean female age in each group was lower than 32 years old, confirming that these outcomes could be related to the capacity of zygotes from young women to partially fix DNA damage, as previously hypothesized.

In contrast, Rodrigo *et al.* (2011) reported a difference in pregnancy rates comparing embryo obtained using sperm from NOA or from OA patients and also noticed a higher rate of aneuploidy in the NOA group detected using the fluorescence *in situ* hybridization (FISH) technique on sperm. However, the sample size analyzed was low: 16, 19 and 10 patients were enrolled in OA, NOA and control groups, respectively. Coates *et al.* (2015) analyzed 3835 embryos deriving from 629 couples, noticing a higher aneuploidy rate in embryos derived from ICSI oligozoospermia cycles compared with standard insemination or normal semen cycles. This difference however was not found in ICSI oligozoospermia cycles using donor eggs, probably because younger eggs have a higher repairing potential bypassing the semen problems. Tsai *et al.* (2015) also found no difference when comparing pregnancy rates from NOA or OA patients and, interestingly, the patients enrolled in the study had a female mean age <33 years old.

In one of the few studies analyzing aneuploidy rates of ICSI versus standard insemination-derived embryos, Munné *et al.* (1998) found that ICSI embryos did not show an increase in aneuploidy rates compared with embryos created using standard insemination, unless the parents had a balanced chromosomal abnormality.

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Table 1. Comparison of general parameters obtained in the four groups

	Group 1 (normal)		Group 2 (OAT)		Group 3 (crypto-spermia)		Group 4 (NOA)		*P*
Cycles	95		58		29		19		
COCs	1347		840		439		305		
MII	1003	74.5%	597	71.1%	332	75.6%	251	82.3%	0.002
Injected	930	92.7%	567	95.0%	305	91.9%	231	92.0%	0.192
Fert	731	78.6%	384	67.7%	213	69.8%	143	61.9%	<0.001[a]
Emb d3	731		384		213		143		
Blast tot	399	54.6%	217	56.5%	125	58.7%	70	49.0%	0.298[b]
Blast d5	248	62.2%	120	55.3%	79	63.2%	31	44.3%	0.018[c]
Blast d6	120	30.1%	84	38.7%	36	28.8%	33	47.1%	0.008[d]
Blast d7	31	7.8%	13	6.0%	10	8.0%	3	4.3%	0.685
Biopsied blastocysts	364	91.2%	209	96.3%	121	96.8%	70	100.0%	0.003[e]
Euploid blastocysts	161	44.2%	93	44.5%	61	50.4%	29	41.4%	0.593
Aneuploid blastocysts	165	45.3%	91	43.5%	49	40.5%	31	44.3%	0.829
Mosaic blastocysts	36	9.9%	22	10.5%	10	8.3%	10	14.3%	0.608
Transfer	76		43		21		14		

[a]Group 1 shows a strongly significant difference vs all the other groups while group 4 show a weekly statistical significant lower fertilization rates.
[b]Groups 3 and 4 show a weekly significant difference ($P = 0.0823$).
[c]Group 4 shows a statistical significant lower rate of blastulation in day 5 compared with all the other groups.
[d]Group 4 shows a statistical significant higher rate of blastulation in day 5 compared with all the other groups.
[e]Groups 1 and 4 show a strongly significant difference ($P = 0.0051$).

Table 2. Statistical analysis with OR of pregnancy rates and abortion rates

	Abortion					Beta				
	n	%	OR	CI 95%	*P*	n	%	OR	CI 95%	*P*
Normal semen	6	7.9	1			44	57.9	1		
NOA	4	28.6	4.67	1.1–19.5	0.035	9	64.3	1.31	0.4–4.3	0.656
Cryptospermia	1	4.7	0.58	0.1–5.1	0.627	16	76.2	2.33	0.8–7.1	0.133
OAT	2	4.7	0.57	0.1–3.0	0.502	30	69.8	1.68	0.8–3.7	0.201
	Clinical					Births				
	n	%	OR	CI 95%	*P*	n	%	OR	CI 95%	*P*
Normal semen	35	46.1	1			26	34.2	1		
NOA	9	64.3	2.11	0.6–6.9	0.216	3	21.4	0.52	0.1–2.0	0.353
Cryptospermia	14	66.7	2.34	0.9–6.5	0.1	10	47.6	1.75	0.7–4.7	0.263
OAT	27	62.8	1.98	0.9–4.2	0.081	22	51.2	2.01	0.9–4.3	0.072

However, in that report, embryos were biopsied on day 3 and analyzed using FISH for chromosomes X, Y, 13, 16, 18, and 21. Only a subset of the karyotype was therefore analyzed, and the use of FISH for determining aneuploidy in human embryos has come under considerable scrutiny due to its limitations for chromosomal screening of preimplantation embryos. Our results have been confirmed by Mazzilli *et al.* (2017) in both fertilization and aneuploidy rates. In their work the genetic health of 1219 blastocysts was analyzed divided in four groups by semen quality. Analogous to our study, they found no differences among the groups in gestational age, birth weight and congenital malformations. However, Mazzilli and collaborators did not define any inclusion criteria, consequently even for couples with female factors that had been enrolled in the study. Despite such differences, our data correlated with their results. Coates *et al.* (2015) analyzed the incidence of chromosomal aneuploidy, finding that chromosomes 1, 2 and 11 were statistically significantly more involved in embryo aneuploidies in homologous ICSI cycles, whereas chromosome 18 was more frequently involved in heterologous ICSI cycles, probably because chromosome 18 alterations are borne by the egg whereas the others are borne by the sperm. They also speculated that chromosomal 18 alterations may be caused by an impaired segregation caused by sperm suboptimal quality.

We performed the same analysis, screening all the aneuploidies divided on the basis of the type of alteration (monosomies, trisomies and complex aneuploidies) without finding any statistical

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difference in the chromosome involved. This outcome could be once more related to the age of the woman. Anyway, we did not find any particular incidence on chromosome 18 other than that alteration is usually borne by women.

Despite the heterogeneity of available data in the literature, the large pool of patients analyzed by our centre were give strong hope of fathering for men who have an extremely low semen quality or even to those who will undergo a testicular biopsy. Nevertheless, it is important to clarify that this outcome could be strictly linked to the female partner age, as oocyte quality seems to be the discriminating factor between success and failure. An interesting result is the higher abortion rate in NOA patients compared with other groups, considering that all the transfers in this study were made only by euploid blastocyst replacements. For this reason, the increased risk of miscarriage could be linked to a late paternal effect of which we are not yet aware.

Our present study is subject to a few limitations. This study had a retrospective approach therefore cannot be used to predict in any way the result of a cycle. Also, chromosomal analysis showed no statistically significant result, probably due to the low number of cycles observed; in fact to effectively analyze the aneuploidy rate on each chromosome a much bigger number of cycles is needed.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S0967199422000119

Author contributions. RP played a central role in the embryologic laboratory procedures, and was involved in the study conception and design, data analysis and drafting the manuscript. MGM is the director of the laboratory, coordinated all the embryological laboratory procedures and was involved in the study conception and design, critical revision of the manuscript and final approval. FS played a central role in the embryologic laboratory procedures, especially in the treatment of testicular samples, data analysis and final approval. BD was involved in the study final review and data analysis. EG was involved in the study conception and design, in the critical revision of the manuscript, in stimulation protocols and final approval.

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Conflict of interest. The authors declare that they have no conflict of interest.

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