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ORIGINAL ARTICLE ***Embryology***

Intracytoplasmic sperm injection is not superior to conventional IVF in couples with non-male factor infertility and preimplantation genetic testing for aneuploidies (PGT-A)

Neelke De Munck[1,*], Ibrahim El Khatib[1], Andrea Abdala[1], Ahmed El-Damen[1], Aşina Bayram[1], Ana Arnanz[1], Laura Melado[1], Barbara Lawrenz[1,2], and Human M. Fatemi[1]

[1]IVIRMA Middle East Fertility Clinic, IVF laboratory, Abu Dhabi, United Arab Emirates [2]Obstetrical Department, Women's University Hospital Tuebingen, Tuebingen, Germany

*Correspondence address. Marina Village, Villa B22/23, Abu Dhabi, United Arab Emirates. E-mail: Neelke.DeMunck@ivirma.com

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STUDY QUESTION: Does the insemination method impact the euploidy outcome in couples with non-male factor infertility?

SUMMARY ANSWER: Conventional IVF can be applied in cycles with preimplantation genetic testing for aneuploidies (PGT-A), as both IVF and ICSI generate equal numbers of euploid blastocysts.

WHAT IS KNOWN ALREADY: Ever since its introduction, the popularity of ICSI has increased tremendously, even in couples with non-male factor infertility. The use of conventional IVF is a contraindication for couples undergoing PGT to ensure monospermic fertilisation and to eliminate potential paternal contamination from extraneous sperm attached to the zona pellucida. Despite this, it has recently been shown that sperm DNA fails to amplify under the conditions used for trophectoderm biopsy samples.

STUDY DESIGN, SIZE, DURATION: This single-centre prospective pilot study included 30 couples between November 2018 and April 2019.

PARTICIPANTS/MATERIALS, SETTING, METHOD: Arab couples, with a female age between 18–40 years, body mass index $\leq$30 kg/m^2, at least 10 cumulus oocyte complexes (COCs) following oocyte retrieval (OR) and normal semen concentration and motility (WHO) in the fresh ejaculate on the day of OR, were eligible for the study. Half of the sibling oocytes were assigned to conventional IVF, and the other half were assigned to ICSI. All embryos were cultured in a time-lapse imaging system in Global Total LP media. Blastocysts were subjected to trophectoderm biopsy on Day 5, 6 or 7 and next-generation sequencing (NGS) to determine blastocyst ploidy status. The primary objective was to determine the euploid rate in blastocysts from sibling oocytes.

MAIN RESULTS AND THE ROLE OF CHANCE: A total of 568 COCs were randomly allocated between IVF ($n = 283$; 9.4 ± 4.0) and ICSI ($n = 285$; 9.5 ± 4.1). While the incidence of normal fertilisation per cycle (6.1 ± 3.8 (64.0%) vs 6.3 ± 3.5 (65.4%); $P = 0.609$) was distributed equally between IVF and ICSI, the degeneration rate (0.1 ± 0.3 vs 0.7 ± 0.8; $P = 0.0003$) was significantly higher after ICSI and the incidence of abnormal fertilisation ($\geq$3 pronuclei) was significantly higher after IVF (0.9 ± 1.2 vs 0.2 ± 0.4; $P = 0.005$). For all fertilised oocytes, there were no differences in the number of good-quality embryos on Day 3 (74% vs 78%; $P = 0.467$), nor in the blastulation rate on Day 5 (80.4% vs 70.8%; $P = 0.076$). The total number of blastocysts biopsied per cycle on Days 5, 6 and 7 was not significantly different between IVF or ICSI (4.0 ± 2.8 vs 3.9 ± 2.5; $P = 0.774$). With euploid rates of 49.8 and 44.1% ($P = 0.755$; OR: 1.05664 [0.75188–1.48494), respectively, there was no significant difference identified between IVF and ICSI (2.0 ± 1.8 vs 1.9 ± 1.7; $P = 0.808$) and all couples had at least one euploid blastocyst available for transfer. When considering only euploid blastocysts, the male/female ratio was 61/39 in IVF and 43/57 in ICSI ($P = 0.063$).

LIMITATIONS, REASON FOR CAUTION: This is a pilot study with a limited patient population of 30 couples (and 568 COCs) with a normal ovarian response. The results of our study should not be extrapolated to other patient populations.

WIDER IMPLICATIONS OF THE FINDINGS: It is safe to apply conventional IVF in couples with non-male factor infertility undergoing PGT-A.

STUDY FUNDING/COMPETING INTEREST(S): No funding was obtained. There are no competing interests.

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Key words: IVF / ICSI / PGT-A / blastocyst / non-male factor

Introduction

Conventional IVF was initially developed for the treatment of female infertility and did not overcome the challenges posed by male factor. While beneficial results can be obtained with high insemination concentrations (HIC) in mild-male factor infertility (Ord *et al.*, 1993; Oehningen *et al.*, 1996; Tournaye *et al.*, 2002), it was only with the introduction of intracytoplasmic sperm injection (ICSI) in 1992 that improved fertilisation outcomes were obtained in couples with male factor infertility (Palermo *et al.*, 1992). Despite the lack of scientific evidence proving benefit in the absence of male factor, the popularity of ICSI has increased exponentially worldwide, with many centres using ICSI almost exclusively as their insemination method (Kupka *et al.*, 2014; Boulet *et al.*, 2015; European IVF-Monitoring Consortium *et al.*, 2016; Chambers *et al.*, 2017). As such, ICSI is now widely used to treat poor ovarian responders, patients of advanced maternal age and patients with previous fertilisation failure with conventional IVF and even couples with unexplained infertility and mild- or non-male factor infertility.

In comparison to ICSI, conventional IVF facilitates a more 'natural' selection of biologically fit sperm. Various concerns have been raised, but not proven conclusively, regarding safety of the ICSI technique (Patrizio, 1995; Bianchi *et al.*, 1996; Watanabe 2018): (i) the choice of sperm based solely on the embryologists' subjective opinion of sperm phenotypic traits with no knowledge of the sperm genetic quality; (ii) physical or biochemical disturbance of the ooplasm or the meiotic spindle; (iii) errors in the selection of the injection site by inaccurate positioning of the injection needle with respect to the meiotic spindle; (iv) injection of biochemical contaminants; and (v) injection of foreign sperm-associated exogenous DNA.

In non-male factor infertility, the use of ICSI has not been conclusively proven to be beneficial for fertilisation and embryo development, as the available literature can be conflicting (Aboulghar *et al.*, 1996; Staessen *et al.*, 1999; Bhattacharaya *et al.*, 2001; Van Landuyt *et al.*, 2005; Eftekhar *et al.*, 2012; Ming *et al.*, 2015; Grimstad *et al.*, 2016; Tannus *et al.*, 2017; Schwarze *et al.*, 2017; Sustar *et al.*, 2019; Farhi *et al.*, 2019). In the absence of conclusive evidence proving a benefit, ICSI is predominantly used to prevent a re-occurrence of total fertilisation failure after conventional IVF in couples with non-male factor infertility. However, equal or increased pregnancy and live birth rates have been ascribed to the use of conventional IVF (Bhattacharaya *et al.*, 2001; Check *et al.*, 2009 and 2011; Eftekhar *et al.*, 2012; Grimstad *et al.*, 2016; Schwarze *et al.*, 2017; Sustar *et al.*, 2019; Drakopoulos *et al.*, 2019). While conventional IVF seems the most appropriate insemination method in non-male factor infertility, the use of ICSI has been recommended by ASRM, SART and ESHRE/PGDIS for all couples pursuing preimplantation genetic testing (PGT), even for couples with non-male factor infertility. The motivation to choose ICSI over conventional IVF is to ensure monospermic fertilisation, to eliminate potential paternal contamination from extraneous sperm attached to the zona pellucida or to prevent the presence of non-decondensed sperm within blastomeres or cumulus cells (Thornhill *et al.*, 2006; ESHRE Capri Workshop group, 2007, PGDIS meeting 2019, Harton *et al.*, 2011; ASRM, 2012; Berger and Baker, 2014). Despite this suggestion, it has recently been shown that sperm DNA fails to amplify under the conditions used for trophectoderm (TE) biopsy samples, opening doors to the use of conventional IVF in PGT cycles (Lynch *et al.*, 2019).

As a result, the use of conventional IVF has been explored in PGT cycles in recent years (Feldman *et al.*, 2017; Şahin *et al.*, 2018; Palmerola *et al.*, 2019). Similar euploidy rates were obtained for IVF and ICSI in PGT for monogenic disorders (PGT-M) on blastomeres (Feldman *et al.*, 2017), and after fluorescent *in situ* hybridisation (FISH) on blastomeres (Şahin *et al.*, 2018) as well as after PGT for aneuploidies (PGT-A) on TE biopsies (Palmerola *et al.*, 2019). However, the retrospective design of these studies and the very limited information available after TE biopsy highlight the need for more in-depth analysis on the use of conventional IVF in PGT-A cycles. Therefore, we performed a prospective pilot study to verify the effectiveness of conventional IVF and ICSI in PGT-A cycles with non-male factor infertility.

Materials and Methods

Approval for this study was obtained from the Ethics Committee of IVIRMA Middle East Fertility Clinic, Abu Dhabi, UAE (United Arab Emirates) (Research Ethics Committee REFA024) and was registered at the ClinicalTrials.gov website (www.clinicaltrials.gov, trial number NCT03708991). A total of 42 couples signed the informed consent form, and 30 of these were randomised following oocyte retrieval (OR): five patients had <10 cumulus oocyte complexes (COCs) retrieved, six patients had insufficient sperm concentration and/or motility and one patient was recruited for a different study as she experienced an IVF failure.

Study design

This prospective pilot study was performed at IVI RMA Middle East Fertility Clinic, Abu Dhabi, UAE, between November 2018 and April 2019. Couples had to fulfil the following inclusion criteria: female age between 18 and 40 years, body mass index (BMI) ≤ 30 kg/m^2, ≥ 10 COCs after OR, all ovarian stimulation protocols, Arab population, PGT-A analysis and fresh ejaculates. Only ejaculates according to the World Health Organization (WHO, 2010) were eligible: $<1 \times 10^6$/ml round cells, concentration $> 15 \times 10^6$/ml, total motility $\geq 40\%$ and progressive motility $\geq 32\%$, with a progressive motility $\geq 65\%$ after capacitation. As a preliminary semen analysis was not performed at the IVIRMA Fertility Clinic for all patients, normal morphology by strict Kruger criteria was not considered. If suboptimal sperm morphology

was noted on the day of OR, patients were excluded from randomisation. Every couple could only be recruited once for the study. If after the OR at least 10 COCs were obtained, low microscope magnification was used to allocate half of these COCs to one dish (Group I) and the other half of the COCs to another dish (Group II). Three hours after the OR, upon denudation, an electronically generated randomisation list was opened to verify the insemination method for Group I and naturally, Group II received the remaining insemination method.

The primary objective was to assess the euploid rate between IVF and ICSI blastocysts. Secondly, the fertilisation, preimplantation development and sex distribution were compared between IVF and ICSI. Fertilisation and abnormal fertilisation were calculated from the number of COCs assigned to a specific group. Embryo quality on Day 3 was calculated from the number of fertilised zygotes. Blastulation rate and blastocyst quality were calculated on the number of embryos undergoing extended culture to Day 5.

Ovarian stimulation protocols

Ovarian stimulation was performed by standard gonadotropin-releasing hormone (GnRH)-antagonist protocols, using recFSH (recombinant follicle-stimulating hormone) or HMG (human menopausal gonadotropin) as stimulation medication. The stimulation medication dose was decided in accordance with ovarian reserve parameters (La Marca and Sunkara, 2014). Final oocyte maturation was achieved by administration of either 5000–10 000 IU of hCG, 0.3 mg of GnRH agonist (triptorelin) or dual trigger (hCG and GnRH-agonist), as soon as ≥3 follicles ≥17 mm were present. OR was carried out 36 h after the trigger for final oocyte maturation. All included patients had a systemic progesterone level on the day of final oocyte of less than 1.5 ng/ml. Oocytes were collected in Quinn's Advantage Medium with HEPES, (SAGE, Målov, Denmark) supplemented with HSA (Vitrolife, Göteborg, Sweden) (HTF-HSA) and washed in Global Total LP medium for fertilisation after which they were cultured at 37°C, 6% CO_2 and 5% O_2 until denudation.

Semen processing

Immediately after the OR, semen samples were obtained by masturbation, allowed to liquefy and prepared within 60 min. Semen was counted in a Makler chamber and processed (capacitated) on a discontinuous density gradient (90/45, Spermient, COOK), followed by centrifugation at 300*g* for 7–15 min. After centrifugation, the pellet was washed twice in HTF-HSA (750*g*, 5 min) and concentrated to a final concentration between 1 and 5 × 10^6/ml, counted in an improved Neubauer. The HTF-HSA-washed sperm sample was kept at room temperature until being used for ICSI, while the sperm for IVF underwent an additional wash with pre-equilibrated (37°C, 6% CO_2) Global Total LP medium for fertilisation (CooperSurgical) (750*g*, 5 min) and were used immediately after preparation.

Insemination and embryo culture

In the ICSI arm, COCs were denuded (Hyase, Vitrolife) 3 h after OR, and mature oocytes were injected 1 h later (Palermo *et al*., 1992). After injection, oocytes were cultured in individual 25-µL drops of Global Total medium (CooperSurgical) in the EmbryoScope time-lapse incubator (Vitrolife) at 37°C, 6% CO_2 and 5% O_2. In the IVF arm, 10 000 progressive motile sperm were added to a 25-µL fertilisation medium (~0.3 × 10^6/ml) and after pre-equilibration, a single COC was added per 25-µL culture drop. Sperm-COCs were incubated overnight at 37°C, 6% CO_2 and 5% O_2 in G185 incubators (K-Systems), and cumulus cells were removed after 17–20 h, prior to the fertilisation check. Afterwards, IVF zygotes were transferred to Global Total medium and further culture was performed in the EmbryoScope time-lapse incubator. On Day 3 of development, the Global Total culture medium was refreshed by removing 20 µl from each well and adding 20 µL overnight-equilibrated Global Total medium. Embryos were cultured until blastocyst biopsy was performed on Days 5–7 of preimplantation development.

On Day 3 of development (68 h post insemination), all normally fertilised oocytes were scored for the number and symmetry of the blastomeres, fragmentation, presence of vacuoles, granulation and multinucleation. Based on these parameters, an embryo quality (EQ) score was assigned to each embryo: EQ1 (excellent), EQ2 (good), EQ3 (moderate) and EQ4 (poor), as previously described by De Munck *et al*. (2015) with a minor adaptation: embryos with >20% fragmentation were included in EQ3 and not in EQ2. On Day 5 of development (116 h post insemination), blastulation rate was assessed by checking the capacitation to cavitate per embryo undergoing extended culture to Day 5. Blastocysts were scored according to the grading system developed by Gardner and Schoolcraft (1999) based on the expansion stage, the number of cells joining the compaction or blastulation and the appearance of the TE and inner cell mass (ICM). On Day 5 of development, as well as at the time of biopsy, blastocysts were graded and assigned to one of four EQ scores: EQ1 (excellent), EQ2 (good), EQ3 (moderate), EQ4 (poor), as previously described (De Munck *et al*., 2015).

Blastocyst biopsy

Blastocyst biopsy was performed in 10-µl drops of HTF-HSA, the blastocyst was fixed with the holding and positioned with a clear view on the ICM at 12 o'clock and the zona pellucida was perforated by three to five laser pulses of 2.2 ms (OCTAX, Herborn, Germany) after which collapse of the blastocyst was induced. Five to 10 TE cells were aspirated in the biopsy pipet followed by three laser pulses to induce an initial cut of the TE cells inside the biopsy pipette and mechanical 'flicking' method to cut the TE cells inside the biopsy pipette; TE biopsies were washed, placed in 0.2-ml PCR tubes containing 2.5 µl PBS and stored at −20°C until further processing.

Ploidy status of blastocysts by NGS

A whole genome amplification (WGA) protocol was performed on all individual samples (PicoPLEX technology by Rubicon Genomics, Inc; Ann Arbor, Michigan, USA). After WGA, library preparation consisted of the incorporation of individual barcodes for the amplified DNA of each embryo. After isothermal amplification and enrichment, sequencing was performed in a 316 or 318 chip using the Personal Genome Machine sequencing (Life-Thermo Fisher, USA). For sequencing analysis and data interpretation, Ion Reporter software was employed. Embryos were diagnosed as euploid or aneuploid. In case of a result indicating mosaicism, the embryo was classified as 'euploid' when the extent of mosaicism was below 30% and as 'aneuploid' when the

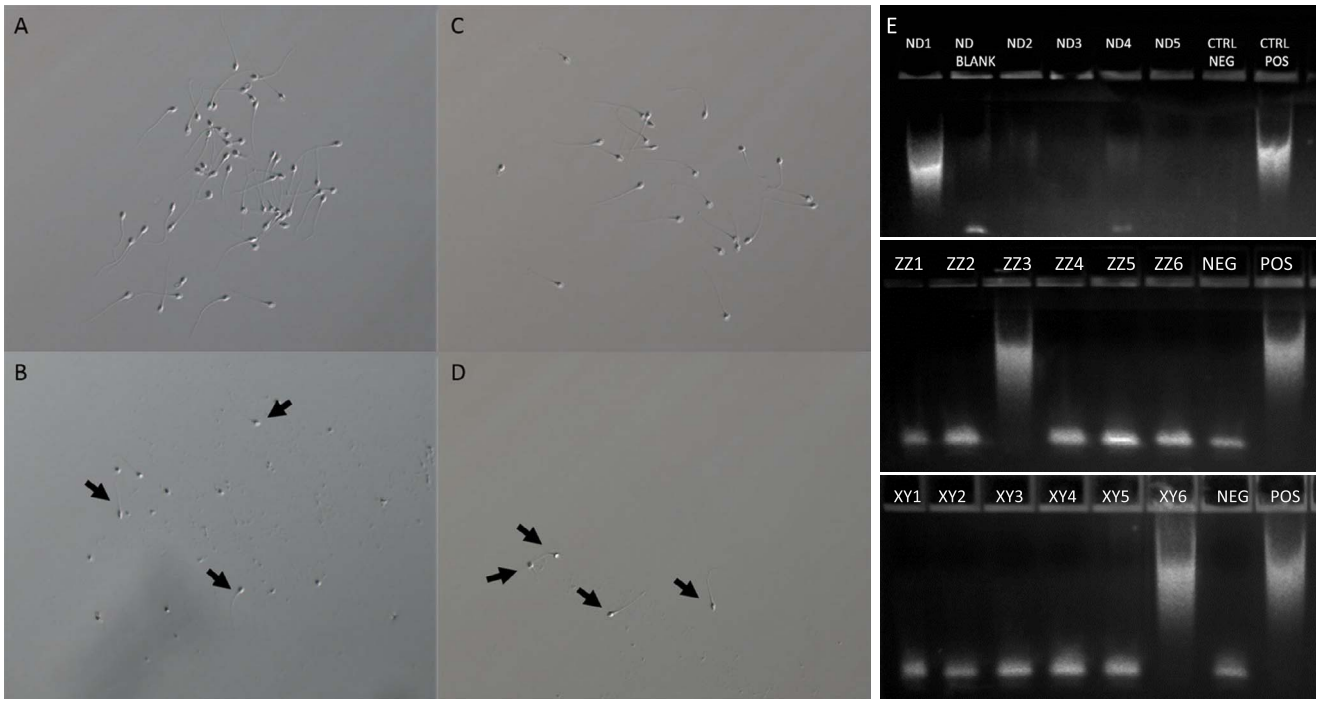

Figure 1 Validation whole genome amplification of sperm. A: sperm cells ($n = 60$) selected for tubing; **B:** sperm cells left in the drop ($n = 3$; arrow) after visual selection of the sperm under the microscope; **C:** sperm cells ($n = 24$) selected for tubing; **D:** sperm cells left in the drop ($n = 4$; arrow) after visual selection of the sperm under the microscope. **E:** WGA results from the three experiments (ND = Experiment 1, ZZ = Experiment 2, XY = Experiment 3); ND1: positive control, ND Blank: negative control, ND2: 60 sperm, ND3: 25 sperm, ND4: 60 sperm, ND5: 25 sperm; ZZ1: 20 sperm, ZZ2: 20 sperm, ZZ3; positive control, ZZ4; negative control, ZZ5: 3 sperm, ZZ6: 10 sperm; XY1: 10 sperm, XY2: 10 sperm, XY3: 3 sperm, XY4: 2 sperm, XY5: negative control, XY6: positive control.

extent of mosaicism was above 30%. Chaotic embryos were defined as those showing a complex pattern of aneuploidies, involving more than six chromosomes. The NGS platform used herein has been validated in previous studies (Wells *et al.*, 2014 and Kung *et al.*, 2015) and is commercially available. Aside from the genetic outcome of the blastocyst, the sex of the embryo was also revealed.

WGA of sperm cells

A triplicate experiment was performed in which 2–60 sperm cells (Fig. 1) were collected in washing solution and tubed under the same conditions as the TE samples. Each experiment contained a positive control (TE sample of an abnormal embryo) and a negative control (blank collected after tubing the positive control) as well as a negative and positive control from Igenomix. An insemination dish with fertilisation medium was prepared and 10 000 progressive motile sperm were added per 25-µl culture drop and cultured overnight as for a normal conventional IVF. On Day 1, the sperm cells were washed in Global Total medium and moved to a clean culture drop of Global Total medium. After 1 day of culture in Global Total medium, motile sperm cells were immobilised with an ICSI needle after which they were transferred with an ICSI needle to a dish with washing solution. The sperm was collected with a 300-µl stripper (COOK) under an inverted microscope to ensure that indeed at least two sperm cells were tubed. By pipetting five times up and down in the washing solution, it was ensured that all sperm cells were tubed; after tubing the stripper was cleaned under the inverted microscope to ascertain no sperm cells were left in the stripper. The WGA protocol, as used for the TE samples and described above, was applied, after which DNA concentrations were measured with Qubit. The threshold for TE samples was set at 40 ng/µl.

Statistical analysis

Continuous variables are presented as mean and standard deviation. Categorical variables are presented as number of observations and percentages.

Percentages were analysed using GLIMMIX procedure to consider the random effect patient (since one patient could have different embryos). The containment method was used to determine the denominator degrees of freedom for tests of fixed effects. The estimation technique used was Residual PL. The response distribution chosen was Poisson and Beta with link function log and logit respectively. The model was retained until the convergence criterion (GCONV = 1E-8) was satisfied, and the estimated G matrix was positive definite. Comparisons were made using procedure PDIFF (*t*-test that is equivalent to the *F*-test) of SAS. Proc GLIMMIX was also chosen because of the capacity of handling unbalanced data. The random effect structure used for this model was variance components (VC), even though there is just one single random effect. Proc MIXED was used to analyse

Table I Sperm characteristics.

Volume (ml)	**2.7 ± 1.2**
Before capacitation	
Total concentration	173.8 ± 138.1
Concentration ($\times 10^6$/ml)	81.5 ± 60.7
Motility (%)	
A	37.0 ± 18.5
B	19.7 ± 16.2
C	5.6 ± 4.8
After capacitation	
Concentration ($\times 10^6$/ml)	2.7 ± 1.1
Motility (%)	
A	74.0 ± 21.2
B	19.5 ± 20.2
C	2.4 ± 1.7

Concentration and motility before and after capacitation. A: rapidly progressive, B: slowly progressive and C: non progressive. Results are expressed as average ± SD.

continuous variables. The same parameters were applied than for proc GLIMMIX.

Interactions were not considered as nested factors since they were not relevant for the model. Quality blastocysts was also analysed with Proc GLIMMIX using Poisson response distribution.

P values, odds ratios and confidence interval at 95% (OR [95% CI]) are presented in the summary tables, in association with the descriptive statistics. A *P* value of 0.05 (both sides) was considered statistically significant. All analyses were performed using SAS studio (SAS® Studio). There were no missing values for any of the collected variables that were analysed.

Results

Thirty patients were recruited for sibling oocyte randomisation in this study, with an average age of 30.3 ± 5.2 [22–39] years old, a BMI of 25.1 ± 3.3 [18.8–29.9] kg/m^2 and AMH levels of 4.2 ± 2.6 [0.85–11.68] ng/ml. Patients were stimulated for 11.5 ± 1.1 days with a total gonadotropin use of 2204.0 ± 640.6 IU. Fifteen patients were triggered with hCG, 10 with a Dual trigger and 5 with GnRHa. Half of the patients (53.3%) experienced primary infertility; the other half (46.7%) had secondary infertility. The major indication for PGT-A was recurrent abortion (47%), while less common indications were advanced maternal age and repeated implantation failure. Male partners were on average 33.2 ± 4.9 [23–44] years old; their abstinence duration ranged from 1 day up to 1 month. Sperm characteristics on day of OR are presented in Table I. The WGA experiment in which 2–30 sperm cells were collected showed that the WGA protocol was unable to amplify sperm DNA (Fig. 1). The highest Qubit value obtained for sperm DNA was 3.26 ng/µl, the negative control was 1.54 ng/µl, the positive control was 25.5 ng/µl and TE samples gave Qubit values above 46.4 ng/µl.

After OR, a total of 568 oocytes were obtained and half were randomly assigned to IVF and the other half to ICSI (9.4 ± 4.0 vs 9.5 ± 4.1; *P* = 0.645) (Table II). No significant difference was observed in the maturation and fertilisation rates. However, degeneration per cycle was higher after ICSI (0.1 ± 0.3 vs 0.7 ± 0.8; *P* = 0.0003) and abnormal fertilisation (≥3PN) was more frequently observed after IVF (0.9 ± 1.2 vs 0.2 ± 0.4; *P* = 0.005). On Day 3 of development, a higher number of EQ1 embryos was observed after ICSI, which was counterbalanced by a higher number of EQ2 embryos after IVF. Of the embryos undergoing extended culture to the blastocyst stage, no difference was observed in the blastulation rate on Day 5 between both insemination methods (8.4 vs 70.8%; *P* = 0.076; OR: 1.10062 [0.99364–1.21919]). Also, the proportion of good- and bad-quality blastocysts was not different between groups (*P* = 0.720). There was no difference in the number of blastocysts biopsied per cycle between IVF and ICSI (4.0 ± 2.8 vs 3.9 ± 2.5; *P* = 0.774), nor in the number of euploid blastocysts (2.0 ± 1.8 vs 1.9 ± 1.7; *P* = 0.808) (Table III); all biopsied blastocysts were informative. The average euploid rate per cycle was not different between conventional IVF and ICSI (49.8 vs 44.1%, *P* = 0.775; OR: 1.05664 [0.75188–1.48494].

Total fertilisation failure was observed in the ICSI arm of one patient; two patients had no blastocysts biopsied from the ICSI arm, while three (10.0%) and six patients (20.0%) had no euploid blastocyst available from the IVF or ICSI arm, respectively (*P* = 0.472). When considering all IVF and ICSI sibling oocytes together, all patients had at least one euploid embryo available for frozen embryo transfer; the proportion of euploid blastocysts available per patient after IVF and ICSI, is depicted in Figure 2. Considering all euploid blastocysts, there was no difference in the distribution of male and female blastocysts between IVF and ICSI (61.0 vs 39.0%; 42.9 vs 57.1%; *P* = 0.063; Table IV).

Discussion

Sibling oocytes from couples with non-male factor infertility were subjected to conventional IVF and ICSI; no significant differences were found in the fertilisation rate, nor in developmental competence. A similar number of blastocysts were biopsied, and the euploid rate was also comparable between both groups.

Data on euploid rates between conventional IVF and ICSI are very limited. Two retrospective studies performed blastomere biopsy followed by PGT-M or FISH (Feldman *et al.*, 2017; Şahin *et al.*, 2018). Both studies were unable to detect differences in euploid rates between conventional IVF and ICSI (41.7 vs 35.2% and 35.0 vs 30.1%, respectively). However, no information on sperm concentration and motility was provided. Palmerola *et al.* (2019) retrospectively compared 75 IVF cycles to 227 ICSI cycles after TE biopsy and NGS and found no difference in euploid rate between the two insemination methods (27.9 vs 30.0%). However, patients for whom the oocytes were subjected to conventional IVF had significantly higher sperm concentration and motility, highlighting the different indications for the selected insemination method between both groups. We were also unable to find differences in the euploid rates between conventional IVF and ICSI (49.8 vs 44.1%). The higher euploid rates observed in our study may be explained by the inclusion of only couples with non-male factor infertility, the young maternal age (30.3 years) and the use of blastocyst biopsy as compared to cleavage stage biopsy (Feldman *et al.*, 2017; Şahin *et al.*, 2018).

Table II Preimplantation development between IVF and ICSI embryos.

Number of cycles/patients	**30**			
Number of COCs retrieved (average ± SD)	**18.9 ± 8.1**			
Total number of COCs retrieved	**568**			
	IVF	ICSI	*P value*	***OR [95%CI]***
Number of COCs assigned (average ± SD)	9.4 ± 4.0	9.5 ± 4.1	0.645	
Total number of COCs assigned (%)	283 (49.8)	285 (50.2)		
Number of mature oocytes (average ± SD)	8.1 ± 3.7	7.8 ± 3.8	0.349	
Total number of mature oocytes (%)	86.7 ± 12.4	82.4 ± 14.4	0.274	1.2632 [0.84227–1.89471]
Fertilization				
Normal fertilisation (average ± SD)	6.1 ± 3.8	6.3 ± 3.5	0.609	
Total number of normally fertilised oocytes (%)	64.0 ± 21.3	65.4 ± 20.6	0.946	0.97825 [0.52147–1.83516]
Degeneration (average ± SD)	0.1 ± 0.3	0.7 ± 0.8	<0.001	
Total number of degenerated oocytes (%)	0.7 ± 2.8	7.7 ± 9.7	<0.001	0.93299 [0.90001–0.96718]
≥3PN (average ± SD)	0.9 ± 1.2	0.2 ± 0.4	0.005	
Total number of abnormally (≥3PN) fertilised oocytes (%)	10.4 ± 14.2	3.0 ± 6.1	0.062	2.07848 [1.18981–3.6309]
Embryo quality on Day 3				
EQ1 (average ± SD)	3.0 ± 2.8	4.1 ± 3.0	0.043	0.7459 [0.56856–0.97856]
EQ2 (average ± SD)	1.5 ± 1.4	0.8 ± 0.9	0.032	1.76 [1.07726–2.87543]
EQ3 (average ± SD)	0.8 ± 0.9	0.8 ± 1.2	0.887	1.04167 [0.59494–1.82383]
EQ4 (average ± SD)	0.8 ± 1.3	0.8 ± 1.6	0.885	0.95833 [0.54092–1.69784]
Total EQ1 (%)	92 (50.3)	122 (64.6)		
Total EQ2 (%)	43 (24.0)	25 (13.2)	0.023	
Total EQ3 (%)	25 (13.8)	24 (12.7)		
Total EQ4 (%)	22 (12.1)	18 (9.5)		
Blastocyst development on Day 5				
Embryos with extended culture (/2PN)	168	176		
Blastulating (% ± SD)	80.4 ± 20.4	70.8 ± 31.4	0.076	1.10065 [0.99364–1.21919]
EQ1 (average ± SD)	1.3 ± 1.5	1.2 ± 1.6	0.732	1.08333 [0.68869–1.70412]
EQ2 (average ± SD)	2.4 ± 2.3	2.4 ± 1.8	0.869	0.97260 [0.70154–1.34840]
EQ3 (average ± SD)	0.7 ± 0.8	1.0 ± 1.4	0.278	0.73333 [0.42303–1.27125]
EQ4 (average ± SD)	1.2 ± 1.2	1.3 ± 1.4	0.650	0.9000 [0.57371–1.41187]
Total EQ1 (%)	39 (23.5)	35 (20.0)		
Total EQ2 (%)	69 (41.6)	73 (41.7)	0.720	
Total EQ3 (%)	22 (13.3)	30 (17.1)		
Total EQ4 (%)	36 (21.7)	37 (21.1)		

Number of oocytes assigned, maturation and fertilisation rate between IVF and ICSI. Results are expressed as average ± SD per cycle (n = 30) or % ± SD per cycle (*n* = 30). Embryo quality on Day 3 and Day 5. COC: cumulus oocyte complex, PN: pronucleus, EQ: embryo quality, SD: standard deviation. Degrees of freedom: 1.

Genetic contamination of the TE samples from maternal cumulus cells and adherent sperm cells has been suggested as possible factors adversely affecting the accuracy of genetic test results (Harton *et al*., 2011; ASRM, 2012; Palmerola *et al*., 2019). Enzymatic removal of cumulus cells, combined with mechanical removal, is not always successful in removing all cumulus cells before ICSI, while mechanical pipetting of IVF oocytes prior to the fertilisation check allows a faster, easier and less invasive removal of all cumulus cells. When TE cells are already herniating at the time of biopsy, the biopsy will be performed away from the zona where potential sperm contamination may happen. In this study, the zona was opened at the time of the biopsy, and the cells were taken with the flicking method. Even though this technique is prone to potential contamination, visual inspection of the TE pieces confirmed the complete absence of sperm. Moreover, it has recently been shown that sperm DNA fails to amplify under the conditions used for PGT-A on TE samples (Lynch *et al*., 2019). These results were also confirmed in this study, as no sperm DNA amplification was observed, even if 60 sperm cells were tubed. Although the immobilised motile sperm were collected on Day 1, the sperm attached to the zona at the time of biopsy are intact and not showing signs of degeneration. Therefore, amplification will also be absent on Day 5 of development, and we suggest that a different protocol should be applied to obtain

Table III Blastocyst biopsy and euploid blastocysts.

	IVF	ICSI	*P value*	OR [95% CI]
Blastocyst biopsy				
Day 5 (average ± SD)	2.7 ± 2.7	2.6 ± 2.1	0.941	
Day 5 (%)	60.1 ± 27.6	55.7 ± 35.5	0.244	0.77769 [0.51768–1.16827]
Day 6 (average ± SD)	1.3 ± 0.8	1.2 ± 1.2	0.758	
Day 6 (%)	37.4 ± 26.9	34.3 ± 32.2	0.846	1.08812 [0.46696–2.53556]
Day 7 (average ± SD)	0.07 ± 0.3	0.03 ± 0.2	NA	
Day 7 (%)	2.5 ± 10.1	3.3 ± 18.3	NA	NA
Total (average ± SD)	4.0 ± 2.8	3.9 ± 2.5	0.774	
Total (%)	67.4 ± 22.1	60.6 ± 29.6	0.619	1.10702 [0.74608–1.64256]
Euploid blastocysts				
Day 5 (average ± SD)	1.4 ± 1.7	1.5 ± 1.4	0.923	
Day 5 (%)	65.9 ± 41.1	64.2 ± 42.2	0.2213	0.67798 [0.41340–1.11190]
Day 6 (average ± SD)	0.53 ± 0.7	0.4 ± 0.6	0.425	
Day 6 (%)	24.0 ± 35.4	15.8 ± 27.9	0.484	1.52163 [0.47714–4.85261]
Day 7 (average ± SD)	0.0 ± 0.0	0.0 ± 0.0	NA	
Day 7 (%)	0 (0.0)	0 (0.0)	NA	NA
Total (average ± SD)	2.0 ± 1.8	1.9 ± 1.7	0.808	
Total (%)	49.8 ± 28.8	44.1 ± 34.6	0.755	1.05664 [0.75188–1.48494]

Total number of blastocysts biopsied and euploid per day of biopsy (5, 6 or 7). Blastocyst biopsy expressed per normally fertilised oocytes; euploid blastocysts expressed per blastocyst biopsied. Results are expressed as average ± SD per cycle ($n = 30$) or % ± SD per cycle ($n = 30$). SD: standard deviation, NA: not applicable. Degrees of freedom: 1.

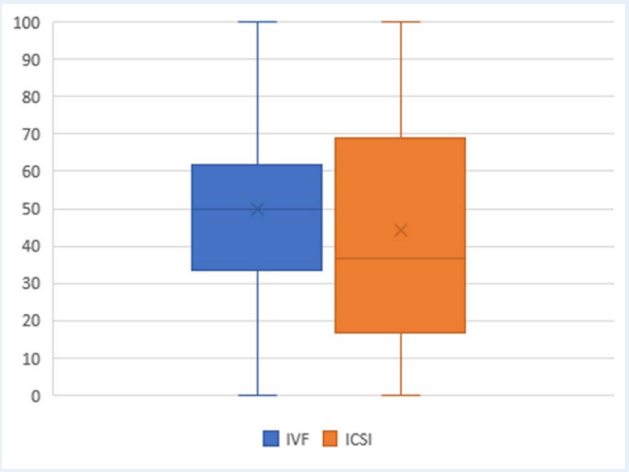

Figure 2 Euploid rate for conventional IVF and ICSI. Figure with average euploid rates for the 30 cycles, min–max, first and third quartiles.

Table IV Sex of euploid and aneuploid embryos.

	IVF *n* (%)	ICSI *n* (%)
Biopsied	120	116
Euploid	59 (49.2)	56 (48.3)
XY	36	24
XX	23	32
Aneuploid	54 (45.0)	55 (47.4)
XY	24	26
XX	26	23
X0	2	3
XXY	2	1
XXX	/	1
-Xq	/	1
Complex abnormal	7 (5.8)	5 (4.3)

XY: male, XX: female, X0: lack of second sex chromosome, XXY: Klinefelter, XXX: trisomy X, –Xq: deletion on the long arm of chromosome X.

sperm DNA amplification. For this reason, the initial assumptions to prevent contamination and defending the use of ICSI can be put aside; the results of this study reassure that conventional IVF can be safely applied for couples with non-male factor infertility or in cases in which oocyte quality is not amenable to ICSI.

As immature oocytes are not selected for ICSI, the maturation rate for ICSI oocytes is scrutinised on the day of OR. However, the maturation rate of IVF oocytes is only verified 16–18 h after the maturation rate for ICSI oocytes, as denudation is only performed on Day 1. As such, a higher maturation rate is expected in IVF siblings as they have the possibility to mature overnight. However, after assigning an equal number of COCs to each group (9.4 ± 4.0 vs 9.5 ± 4.1), the maturation rates were not different between IVF (8.1 ± 3.7) and ICSI (7.8 ± 3.8). When analysing the fertilisation potential in our cohort of patients with non-male factor infertility, we were unable to detect any

significant difference between IVF and ICSI sibling oocytes in terms of normal fertilisation; 6.1 ± 3.8 vs 6.3 ± 3.5. These results are in line with previous studies on couples with non-male factor infertility in which equal fertilisation rates were observed between both insemination methods on sibling oocytes (Ruiz et al., 1997; Staessen et al., 1999; Van Landuyt et al., 2005). However, Ming et al. (2015) observed higher fertilisation rates after IVF on sibling oocytes in couples with non-male factor infertility, while others have shown higher fertilisation rates after ICSI on sibling oocytes (Khamsi et al., 2001; Farhi et al., 2019). While sibling oocyte studies facilitate exclusion of maternal factors that may influence developmental competence, prospective randomised controlled trials, matched controlled trials and large retrospective studies in couples with non-male factor infertility have also shown equal fertilisation rates or fertilisation rates favouring IVF (Aboulghar et al., 1996; Hsu et al., 1999; Bhattacharaya et al., 2001; Foong et al., 2006; Kim et al., 2007; Ou et al., 2010; Eftekhar et al., 2012; Tannus et al., 2017; Schwarze et al. 2017; Li et al., 2018; Sustar et al., 2019; Drakopoulos et al., 2019).

Fertilisation failure after conventional IVF is much lower in couples with non-male factor infertility (~10%) compared to couples with mild-male factor infertility (45%) (Aboulghar et al., 1996; Verheyen et al., 1999; Plachot et al., 2002; Tournaye et al., 2002; Kihaile et al., 2003). A large retrospective study in Latin America, including almost 50 000 IVF or ICSI cycles in couples with non-male factor infertility, demonstrated a lower fertilisation failure rate after conventional IVF (3.37 vs 4.49%) (Schwarze et al., 2017), and this concurs with many other studies demonstrating equal or lower fertilisation failure rates after conventional IVF with normozoospermia (Kim et al., 2007; Tannus et al., 2017). However, some older studies have found a higher fertilisation failure after IVF (Ruiz et al., 1997; Staessen et al., 1999). The fact that some studies suggest that fertilisation rates are higher with IVF in circumstances of normozoospermia may be attributed to the fact that ICSI oocytes are more prone to degeneration due to mechanical damage, which is not the case for oocytes inseminated by IVF. This damage may be induced during the denudation process as well as during the ICSI procedure itself. The enzymatic and mechanical stress during the removal of cumulus cells may cause a high degree of spindle deviations prior to the ICSI procedure (Hewitson et al., 1999; Rienzi et al., 2003); these are generally not observed after IVF, which allows a gentle and fast removal of the cumulus cells on Day 1, especially if the oocyte is fertilised. During the ICSI procedure itself, mechanical damage to the oocyte may lead to degeneration, with operator variabilities ranging from 5 to 19% (Ebner et al., 2001). Indeed, the degeneration rate after ICSI was significantly higher in our pilot study ($P = 0.0003$); however, this was counterbalanced by a higher abnormal fertilisation rate after IVF ($P = 0.005$). Even though a progressively motile sperm concentration of 0.33×10^6/ml was used, which is within the advised ESHRE guidelines (2015) of $0.1–0.5 \times 10^6$/ml, the 9.5% abnormal fertilisation rate observed in this study is above the competency levels of the Vienna consensus (ESHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive Medicine, 2016). Reducing the insemination concentration to the lower reference limit of 0.1×10^6/ml might be beneficial for the normal fertilisation rate and hence also for the blastocyst development after conventional IVF. While no further options are available after fertilisation failure in ICSI oocytes, a rescue-ICSI procedure can be applied on failed-fertilised IVF oocytes; not only will this technique increase the fertilisation rate for the patient, they also moderately increase pregnancy rates (Morton et al., 1997; Yuzpe and Fluker, 2001). With different screening tests available and every stimulation cycle being different, no test will give a 100% guarantee to prevent fertilisation failure; not after conventional IVF and not after ICSI (Mahutte and Arici, 2003).

Since the introduction of ICSI to the present day, developmental competence has been extensively studied between conventional IVF and ICSI. Particularly, in couples with male factor infertility, negative selection during preimplantation development is attributed to the selection of abnormal sperm for ICSI which may result in abnormal cleavage and developmental arrest (Shoukir et al., 1998; De Vos et al., 2003). This paternal effect becomes more pronounced after embryonic genome activation around Day 3, which is translated in an increased developmental arrest to the blastocyst stage in ICSI embryos (Miller and Smith, 2001). In couples with non-male factor infertility, sperm velocity is sufficient for cumulus-zona penetration in IVF and rapidly progressive, morphologically normal sperm are selected for ICSI. This translates into equal or improved blastocyst formation following conventional IVF (Ruiz et al., 1997; Staessen et al., 1999; Khamsi et al., 2001; Jeziorowski et al., 2002; Van Landuyt et al., 2005; Ming et al., 2015; Tannus et al., 2017). Conventional IVF is an appropriate option for a young population with unexplained or tubal infertility with a normal response to ovarian stimulation. The same is also true of patients with an extremely low ovarian response and those of advanced maternal age (Ou et al., 2010; Sfontouris et al., 2015; Tannus et al., 2017; Schwarze et al. 2017; Guo et al., 2018; Liu et al., 2018; Farhi et al., 2019). These results are in keeping with the development observed in this study; no significant differences were observed in the number of blastulating embryos on Day 5 (80.4 vs 70.8%; $P = 0.076$) and EQ on Day 5 ($P = 0.720$). On completion of blastocyst culture, an equal number of blastocysts were biopsied in both the IVF and ICSI study groups (4.0 ± 2.8 vs 3.9 ± 2.5; $P = 0.774$).

While this is a pilot study with a limited study group, this is the first prospective trial to verify the euploid rate in IVF/ICSI sibling oocytes and demonstrates that IVF will not jeopardise a couple's cycle; the absence of euploid embryos was observed in 3/30 (10%) IVF and 6/30 (20%) ICSI cycles. Even though ICSI is popular to salvage fertilisation failure, this study experienced only one total fertilisation failure, in the ICSI arm of the study. Sperm morphology was not evaluated before insemination, which could be viewed as a potential bias. As the zona provides a barrier for abnormal sperm, this could only be beneficial for the IVF-inseminated oocytes. With the delivery of a healthy child being the endpoint of every cycle, the inclusion of only 30 cycles provides insufficient data to comment on pregnancy and live birth rates in this study. An ongoing RCT, by Vuong and colleagues, will soon provide data on the effectiveness of IVF and ICSI on live birth rates (NCT03428919) (Dang et al., 2018). As this patient population was on average 30.3 years old, the results cannot be generalised to an older IVF population, even though conventional IVF is highly efficient in patients with advanced maternal age and low ovarian reserve (Liu et al., 2018). In case a non-inferiority trial would be performed based on the outcomes described in this study, a total of 426 blastocysts need to be biopsied in both arms to prove that the euploid rate is equal between conventional IVF and ICSI. A final important consideration is that IVF incurs a significant reduction in cost as compared to ICSI and is a less complex technique for embryologists.

To conclude, this is the first prospective trial comparing the developmental competence and ploidy outcome in patients with non-male factor infertility following conventional IVF and ICSI on sibling oocytes. No significant difference was identified in fertilisation rates, embryo development and number of euploid embryos resulting from either conventional IVF or ICSI. In cases of non-male factor infertility, there is no indication to perform ICSI for all oocytes.

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Authors' roles

N.D.: conception and design of the study; N.D., I.E., A.A., A.D., A.B., A.Ar., L.M., B.L., H.F.: oocyte retrieval; N.D., I.E., A.A., A.D., A.B., A.ar.: IVF and ICSI procedure and embryo evaluations; N.D., I.E., A.A., A.D., A.B., A.ar.: blastocyst biopsy and tubing; N.D.: construction of the database and data interpretation; N.D.: drafting the manuscript; N.D., I.E., A.A., A.D., A.B., A.ar., L.M., B.L., H.F.: critical review and final approval of the manuscript.

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Conflict of Interest

There are no conflicts to declare.

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