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GAMETE BIOLOGY

PICSI vs. MACS for abnormal sperm DNA fragmentation ICSI cases: a prospective randomized trial

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Abstract

Purpose To know which sperm selection technique, physiological intracytoplasmic sperm injection (PICSI) or magnetic-activated cell sorting (MACS), is better for the selection of sperm with abnormal sperm DNA fragmentation (SDF) in patients undergoing intracytoplasmic sperm injection (ICSI).

Methods A prospective randomized trial included 413 ICSI cases with abnormal SDF (> 20.3%) by TUNEL assay. Patients with at least 1 million total progressive motile sperm count were randomized to PICSI or MACS groups on the day of ICSI. PICSI depends on the hyaluronan binding of better SDF sperm where individual sperm was selected, while MACS selects non-apoptotic sperm population using Annexin V magnetic beads. All pre-implantation embryogenic parameters were observed and the main outcome was the ongoing pregnancy rate.

Results There were no significant differences between patients allocated to PICSI and MACS in the studied parameters including pre-implantation embryological data, implantation, clinical pregnancy, and ongoing pregnancy rates. Meanwhile, sub-analysis according to the female age has shown that female patients with less than 30 years of age in the MACS group had significantly higher good-quality blastocyst, clinical pregnancy, and ongoing pregnancy rates than the PICSI group. However, the higher implantation ($p = 0.051$), clinical pregnancy ($p = 0.078$), and ongoing pregnancy ($p = 0.097$) rates observed in females between 30 and 35 years of age in the PICSI group did not reach significance level.

Conclusions PICSI and MACS are efficient techniques for sperm selection in cases with abnormal sperm DNA fragmentation. However, MACS is preferred when the females are younger than 30 years, while PICSI is preferred in older females.

Clinical trial registration number NCT03398317 (retrospectively registered)

Keywords PICSI · MACS · Sperm selection · Sperm DNA fragmentation · ICSI

Introduction

The number of couples seeking infertility treatment is steadily increasing [1]. Infertility affects about 10–15% of couples worldwide [1] and 30–50% of the cases show a paternal cause of infertility which may negatively affect the reproductive outcomes [2, 3]. The management of male infertility can be through treating the underlying causes or using a suitable assisted reproductive technique like intracytoplasmic sperm injection (ICSI) [2]. One of the main key determinants of ICSI outcome is the quality of the embryo that develops after the fertilization of oocytes with poor-quality sperm from infertile men [4]. Conventional semen analysis is insufficient to predict the male fertility potential or the success rate of ICSI [3, 5, 6]. Sperm DNA fragmentation (SDF) is suggested to be a major factor of male infertility [7–9] that negatively affects fertilization [10], cleavage [10], blastulation [9, 10], implantation [11], clinical pregnancy [8, 9], miscarriage [11–13], and live birth rates [7] after in vitro fertilization (IVF) and ICSI. On the other hand, there are indications that oocytes can repair sperm DNA damage to a certain extent [14, 15] depending on the oocyte quality, degree of sperm DNA fragmentation, and time needed for repair [15].

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In vivo, sperm selection in the female genital tract starts from the male germ cells being ejaculated into the upper part of the vagina when the sperm have to move out the seminal plasma, penetrate the cervical mucus, and move up the female genital tract to meet the oocyte for fertilization in the ampulla of the fallopian tube [16, 17]. This extremely stringent selection process aims to select the most capable sperm with high DNA integrity, maturity, and yields to successfully fertilize oocytes and produce a good-quality embryo [16, 18]. Several natural selection mechanisms can discriminate and select the best-quality spermatozoa based on their characteristics [16]. However, in ICSI, all natural barriers for sperm selection, mainly the cumulus cell layer, the zona pellucida, and the oolemma, are bypassed by injecting a single sperm into the oocyte. In turn, bypassing these physiological selection barriers can impair fertilization and embryo development [17]. Currently, the used sperm preparation techniques like density gradient centrifugation (DGC) and swim-up depend on centrifugation and migration of the sperm [19], and have not been evaluated enough to assess SDF's impact on the clinical outcomes [19]; also, they may include a centrifugation process that can negatively affect SDF [20]. Therefore, several advanced sperm selection techniques are developed to mimic the natural selection mechanisms [17, 19]; out of these techniques, we chose (I) elimination of apoptotic sperm (magnetic-activated cell sorting—MACS) and (II) membrane maturity (physiological ICSI—PICSI) [21, 22].

MACS is an effective tool to remove apoptotic sperm from a sperm population [23, 24]. Annexin V specifically binds to phosphatidylserine, which is externalized from the inner membrane leaflet to the outer membrane leaflet if the membrane structure is compromised, DNA integrity decreased, and cells are labeling for abortive apoptosis [23]. Annexin V–conjugated magnetic microbeads are used for magnetic separation using MACS columns and separators yield a sperm population with a lower incidence of apoptosis, higher nuclear maturity [23, 24], higher count and lower SDF [25]. Some studies showed improved fertilization, implantation rates, and clinical pregnancy [26–28]. MACS can be done alone or combined with DGC for extra selection and better reproductive outcomes [23, 24, 28, 29].

Sperm selection using PICSI is based on the fact that only a mature sperm head can bind to hyaluronan (HA), which is a major component of cumulus oophorus and participates in the natural sperm selection events [16]. Hyaluronan-bound sperm are thought to reflect higher maturity, acrosome activity, as well as lower aneuploidies and SDF [30, 31]. Hence, it is thought to improve successful pre-implantation embryogenesis [19, 31, 32]. PICSI as a method of sperm selection is highly specific and has minimal safety concerns [30].

The objective of this study was to determine which method, PICSI or MACS, is better as a sperm selection technique for patients with abnormal SDF undergoing ICSI in terms of their clinical and pre-implantation embryogenic parameters.

Materials and methods

Study design and population

This study was approved by the Ethics Committee of Ganin Fertility Center, Cairo, Egypt, for registration on www.clinicaltrials.gov. Written consents were obtained from patients prior to their inclusion in the study. A total of 413 patients attending Ganin Fertility Center from January 2017 to March 2019 were included in the study for autologous ICSI cycles. Patients were included in the study if the female age was between 18 and 35 years and a minimum of 5 mature oocytes were aspirated for injection. The male partners had to have abnormal sperm SDF (cutoff value is 20.3%, calculated according to the Egyptian population ROC study [33]). The total progressive motile sperm count had to be at least 1 million for the patient to be suitable for any of the used sperm selection techniques. The exclusion criteria included the presence of leukocytospermia, varicocele, or any factors that affect ovarian stimulation or embryo implantation, and a known genetic disorder. Patients were randomized on the day of ICSI using computer software into two groups: the first group: PICSI ($n = 200$); and the second group: MACS ($n = 196$); all participants and investigators except for embryologists were masked to treatment allocation. The primary outcome measures the ongoing pregnancy rate (20 weeks of gestation), while the secondary outcomes include cleavage rate (day 3), blastulation rate (day 5 or 6), good-quality blastulation (day 5 or 6), clinical pregnancy (14 days after embryo transfer), and the implantation rate (6 weeks of gestation).

Sperm DNA fragmentation test

Sperm DNA fragmentation (SDF) was tested twice using the TUNEL assay 3 weeks prior to ICSI procedure. Semen samples were collected after 1 to 2 days of sexual abstinence. Following liquefaction for 30 min, semen samples underwent a microscopic examination to evaluate sperm concentration, motility, and normal morphology according to WHO (2010) criteria.

In preparation for sperm DNA fragmentation (SDF) assessment, an aliquot of liquefied semen containing 5 million sperm was washed in 1 ml phosphate-buffered saline (PBS; Lonza, Verviers, Belgium) at 300×*g* for 4 min. The supernatant was discarded and the pellet fixed by re-suspension in 1 ml 3.7% paraformaldehyde (Sigma-Aldrich, Steinheim, Germany) for 30 min at 4 °C. Paraformaldehyde was removed by centrifugation at 300×*g* for 4 min at room temperature, the supernatant was discarded, and the cells in the pellet permeabilized by adding 1 ml of ice-cold 70% ethanol (CHEM-LAB, Verviers, Belgium) for at least 30 min at − 20 °C.

SDF was determined by means of the TUNEL assay kit Apodirect (BD Pharmingen, San Diego, CA) using a BD Accuri C6 bench-top flow cytometer (BD Pharmingen, San Diego, CA). Positive and negative kit controls provided by the manufacturer and internal controls (semen samples with known DNA fragmentation) were included in each run. All samples were washed twice with washing buffer containing 0.05% sodium azide by centrifugation at 300×*g* for 5 min, followed by adding 50 μl of freshly prepared staining solution containing reaction buffer, FITC-dUTP, and TdT enzyme, followed by a 1-h incubation at 37 °C. Subsequently, the excess staining solution was removed by adding 1 ml rinsing buffer containing 0.05% sodium azide followed by centrifugation at 300×*g* for 5 min and discarding of the supernatant. The pellet was then re-suspended in 0.5 ml propidium iodide (PI)/RNase Staining Buffer (5 μg/ml, 200 μg/ml RNase) and incubated for 30 min at room temperature, followed by flow cytometric analysis. For each sample, 10,000 spermatozoa were counted and evaluated for sperm DNA fragmentation by the "Accuri C flow plus" flow cytometer software (BD Pharmingen, San Diego, CA).

Controlled ovarian stimulation and oocyte pick up

The long protocol was preferred for ovulation stimulation in all study cases, starting on day 3 of the menstrual cycle with combined oral contraceptive pills Gynera® (Bayer, Leverkusen, Germany) for 12–21 days, and 20 IU of Lucrin® (Abbvie, Madrid, Spain) with an overlap of the last 5 days of Gynera®. From day 3 of the next cycle, downregulation was confirmed by endocrine profile and transvaginal ultrasound. When confirmed, ovarian stimulation was started using 150 IU Gonal-F® (Merck Serono, Rome, Italy) or 150 IU Fostimon® (IBSA, Collina d'Oro, Switzerland), combined with 75 IU Menopur® (Ferring, Kiel, Germany). These doses were adjusted in the following days according to each patient's response. When at least two follicles reached a mean diameter of 19 mm or more, 10,000 IU of hCG (Pregnyl®; Organon, Oss, The Netherlands) was intramuscularly injected.

Thirty-six hours after the hCG injection, oocytes were retrieved by ultrasound-guided transvaginal follicle aspiration under general anesthesia. Cumulus-oocyte complexes were collected in multipurpose handling medium MHM (Irvine Scientific, CA, USA) supplemented with 10% serum substitute supplement (SSS; Irvine Scientific). Cumulus-oocyte complexes were washed and cultured in 50-μl drops of continuous single culture (CSC; Irvine Scientific) medium supplemented with 10% SSS for denudation and ICSI procedure.

Sperm preparation and selection

All used sperm preparation and selection techniques were tested before and do not have any adverse or unintended effects that may affect the trial outcomes.

Density gradient centrifugation

All semen samples were subjected to a double-layer density gradient centrifugation using Isolate® (Irvine Scientific) with 1 ml of liquefied semen overlaying 50% and 90% discontinuous isolate layers in a 15-ml conical tube. Samples were centrifuged at 250×*g* for 8 min at room temperature. After centrifugation, the supernatant was aspirated with a Pasteur pipette and the resulted soft pellet washed using Sperm wash medium (Irvine Scientific) and centrifuged again at 250×*g* for 8 min at room temperature. The final pellet was re-suspended in the residual volume to be ready for any of the assigned sperm selection techniques.

Physiological ICSI

PICSI® dishes (Origio, Malov, Denmark) were prepared by hydrating the hyaluronan microdots with 10 μl of MHM medium with 10% SSS. Drops of 10% polyvinylpyrrolidone (PVP) (Irvine Scientific) were placed elsewhere in the dish for sperm immobilization. Few drops of MHM medium were placed for oocyte injection. The dishes were covered with 3–4 ml light mineral oil (Irvine Scientific) and incubated 10 min at 30 °C for optimum sperm binding.

Magnetic-activated cell sorting

To perform MACS® (Miltenyi Biotec, Bergisch Gladbach, Germany), the pellet after DGC was re-suspended and incubated with 60-μl Annexin V–conjugated microbeads and 140 μl 1× binding buffer, followed by 20-min incubation at room temperature in the dark. During the incubation, the MiniMACS column (Miltenyi Biotec) was hanged on the magnetic separator attached to the MACS stand and rinsed with 0.5 ml 1× binding buffer to ensure optimum sperm binding. The sample was loaded onto the column and the eluted portion was collected and evaluated for sperm count and motility.

ICSI, embryo culture, and transfer

For every patient, all metaphase II (MII) oocytes were injected 2–4 h post oocyte pick up and then cultured in 25-μl drops of CSC medium supplemented with 10% SSS in an incubator at 6% Co_2, 5% O_2, and 37 °C. According to the number and quality of the cleaved embryos, the patient was set to transfer on either day 3 or day 5. On day 5, embryos were morphologically evaluated according to criteria described by Gardner [34], where high-quality blastocyst are AA, AB, BA, and BB regardless of their expansion degree. The blastulation rate is calculated as the percentage of blastocysts formed on day 5 or 6 from the number of cleaved embryos. Embryo transfer was

performed using a Wallace® embryo transfer catheter (Origio) and abdominal ultrasound guidance.

Statistical analysis

As considering the primary outcome, sample size calculation was done using the comparison of the ongoing pregnancy rate between MACS and PICSI groups. Calculations were performed based on comparing 2 proportions from independent samples in a prospective study using the chi test, the α-error level was fixed at 0.05, the power was set at 80%, and the group's ratio was set at 1. According to our pilot study, the minimum optimum sample size should be 200 participants in each arm to be able to reject the null hypothesis with the assumed power. The calculation was done using PS Power and Sample Size Calculations software, version 3.0.11 for MS Windows.

All statistical calculations were done using IBM SPSS (Statistical Package for the Social Sciences; IBM Corp, Armonk, NY, USA) release 22 for Microsoft Windows. Results are described in terms of mean ± standard deviation (± SD) or frequencies (number of cases) and percentages when appropriate. A comparison of numerical variables between the study groups was done using Student *t* test for independent samples. For comparing categorical data, chi-square (χ^2) test was performed. A *p* value less than 0.05 was considered statistically significant. A comparison of numerical variables between male age groups was done using a one-way ANOVA test.

Results will include sub-analysis according to the male age (less than 35 years, 36 to 41 years, and older than 41 years) and female age (less than 30 years and from 30 to 35 years) categories as described in previous studies [35–37].

Results

Between January 2017 and March 2019, 413 couples were randomly assigned to receive PICSI or MACS. Seventeen couples were excluded after randomization for mismatching the inclusion criteria; 10 couples had leukocytospermia on the day of ICSI, and 7 produced a smaller number of MII oocytes. Thus, 396 couples were included in the analysis PICSI (n = 200) and MACS (n = 196).

There were no significant differences between the PICSI and MACS groups in female age, male age, primary/secondary infertility, smoking status, sexual abstinence days, SDF, sperm count, number of mature oocytes, and MII ratio. However, the total motility was significantly higher in the PICSI group (Table 1).

In the PICSI group, 26 couples vitrified all of their embryos, 15 couples had day 3 ET, and 159 couples had day 5 ET. In the MACS group, 33 cases vitrified all of their embryos, 10 cases had day 3 ET, and 153 cases had day 5 ET. The average number of embryos transferred per case was 2.28 ± 0.87 in the PICSI group and 2.33 ± 0.81 in the MACS group (p = 0.5873).

The primary analysis showed that the ongoing pregnancy rate of the PICSI group was 55.7% vs. 56.4% for the MACS group (p = 0.8972) (Table 2). The pre-implantation parameters of cleavage, blastulation, and good-quality blastulation rates revealed no differences between both groups (Table 2). The clinical pregnancy rate was 62.4% and 62.1% for PICSI and MACS groups (p = 0.9548), respectively. In addition, implantation rates also showed no difference (p = 0.9553).

While in the PICSI group one case had a biochemical pregnancy, two cases were recorded in the MACS group. Forty-eight cases in the MACS group had multiple pregnancies (44 twins and 4 triplets), whereas 53 multiple pregnancies (52 twins and 1 triplet) were recorded in the PICSI group.

Sub-analysis according to the male age included 3 groups (less than 35 years, 36 to 41 years, and older than 41 years) (Table 3). This comparison showed no differences between PICSI and MACS in any of the studied parameters (Table 3). In addition, comparisons of the 3 male age groups using PICSI technique did not show any difference in cleavage (p = 0.623), blastulation (p = 0.936), good-quality blastulation (p = 0.925), clinical pregnancy (p = 0.528), implantation (p = 0.617), and ongoing pregnancy rates (p = 0.891). Moreover, comparisons of the 3 male groups using MACS also did not show differences in cleavage (p = 0.244), blastulation (p = 0.071), good-

Table 1 Patient characteristics

	PICSI (n = 200)	MACS (n = 196)	p value
Types of infertility			
Primary (%)*	57.3	63.4	0.31
Secondary (%)*	42.6	36.5	
Male partner			
Male age (years)	35.9 ± 6.2	36.2 ± 7.3	0.56
Current smokers (%)*	40.1	30.6	0.15
SDF (% ± SD)	28.5 ± 7.1	28.6 ± 7.2	0.9
Sperm count (× 10^6/ml)	28.7 ± 21.6	31.2 ± 20.1	0.23
Total motility (% ± SD)	50.9 ± 15.8	46.1 ± 14.9	0.002
Sexual abstinence (days)	1.76 ± 0.6	1.81 ± 0.6	0.67
Female partner			
Female age (years)	30.1 ± 4	29.8 ± 3.9	0.45
MII oocytes/case	15.1 ± 8	15.3 ± 8.4	0.81
MII (%)*	82.7 ± 13.4	82 ± 12.6	0.63

Data are mean (SD) unless otherwise stated. *n* refers to the number of patients for whom data were available. *PICSI* physiological intracytoplasmic sperm injection, *MACS* magnetic-activated cell sorting, *SDF* sperm DNA fragmentation, *MII* metaphase II oocytes. *p* values < 0.05 are considered significant

*Data are percentages only

Table 2 Primary analysis of pre-implantation embryological and clinical parameters comparing PICSI and MACS groups

	PICSI ($n = 200$)	MACS ($n = 196$)	p value
Primary outcome			
Ongoing pregnancy (%)*	55.7%	56.4%	0.89
Secondary outcomes			
Cleavage rate (% ± SD)	75.6 ± 17.3	74.7 ± 17.4	0.64
Blastulation rate (% ± SD)	64.3 ± 18.2	63.4 ± 18.8	0.62
Good-quality blastulation rate (% ± SD)	63.7 ± 24.4	66.3 ± 29.3	0.31
Clinical pregnancy rate (%)*	62.4%	62.1%	0.95
Implantation rate (%)*	40.3%	40.0%	0.95

Data are mean (SD) unless otherwise stated. *n* refers to the number of patients for whom data were available. *PICSI* physiological intracytoplasmic sperm injection, *MACS* magnetic-activated cell sorting. p values < 0.05 are considered significant

*Data are percentages only

quality blastulation ($p = 0.396$), clinical pregnancy ($p = 0.181$), implantation ($p = 0.187$), and ongoing pregnancy rates ($p = 0.173$).

Another sub-analysis was done where cases in each group were categorized according to the female age (less than 30 years and from 30 to 35 years) (Table 4). There was no significant difference in SDF values between PICSI and MACS group neither when the female age was less than 30 years nor from 30 to 35 years (Table 4). There was a significant difference in the ongoing pregnancy rate of 51.3% vs. 69.5% for PICSI and MACS groups, respectively ($p = 0.015$), in the same female age group < 30 years (Table 4). Moreover, the MACS group had significantly higher good-quality blastulation and clinical pregnancy rates (Table 4). The analysis of females aged between 30 and 35 showed no significant difference in all of the studied parameters between PICSI and MACS, but the PICSI group showed values near to be significant implantation ($p = 0.051$), clinical pregnancy ($p = 0.078$), and ongoing pregnancy rates ($p = 0.09$) (Table 4). Upon comparing different female age groups using the same technique, we found no significant differences in any of the compared parameters after using PICSI as a sperm selection. However, MACS in females age less than 30 years showed significantly higher good-quality blastulation ($p = 0.04$), clinical pregnancy ($p = 0.003$), implantation ($p = 0.02$), and ongoing pregnancy rates ($p = 0.002$) compared with the female age group from 30 to 35 years.

Table 3 Trial outcomes analyzed according to the male age (years)

	Male age < 35			Male age 36–41			Male age > 41		
	PICSI ($n = 110$)	MACS ($n = 103$)	p value	PICSI ($n = 59$)	MACS ($n = 62$)	p value	PICSI ($n = 31$)	MACS ($n = 30$)	p value
Female age (years)	28.6 ± 3.4	27.8 ± 3.5	0.12	31.4 ± 3.2	31.8 ± 2.9	0.47	32.6 ± 3.6	32.2 ± 2.8	0.63
SDF (%)	27.6 ± 6.8	29 ± 7.5	0.15	28.6 ± 6.2	28.4 ± 6.7	0.86	31.4 ± 8.5	28.1 ± 6.4	0.09
MII	16.2 ± 8.4	15.7 ± 7.6	0.65	13.7 ± 7.5	14.9 ± 8.4	0.41	13.5 ± 6.9	15 ± 10.9	0.52
Primary outcome									
Ongoing pregnancy rate (%)*	58.5	63.9	0.46	55.7	50	0.55	53.5	40	0.32
Secondary outcomes									
Cleavage rate (% ± SD)	75.8 ± 16	75.3 ± 15.7	0.81	74.2 ± 17.6	76.6 ± 15.8	0.43	78.5 ± 21.3	68.4 ± 24.1	0.08
Blastulation rate (% ± D)	64.4 ± 18	63.2 ± 19.7	0.64	63.8 ± 19.1	67 ± 15.3	0.31	65.3 ± 18.2	56.9 ± 21.2	0.10
Good-quality blastulation rate (% ± SD)	63.5 ± 25.2	69.4 ± 24.3	0.08	64.7 ± 23	65.8 ± 30.3	0.82	62.7 ± 25.2	59.5 ± 39.5	0.70
Clinical pregnancy rate (%)*	65.9	68.6	0.70	59.6	59.2	0.97	57.1	47.8	0.50
Implantation rate (%)*	45.9	47.9	0.77	44.4	42.6	0.84	36.4	30.8	0.64

Data are mean (SD) unless otherwise stated. *n* refers to the number of patients for whom data were available. *PICSI* physiological intracytoplasmic sperm injection, *MACS* magnetic-activated cell sorting, *SDF* sperm DNA fragmentation, *MII* metaphase II oocytes. p values < 0.05 are considered significant

*Data are percentages only

Table 4 Trial outcomes analyzed according to female age

	F age < 30			F age 30–35		
	PICSI ($n = 83$)	MACS ($n = 86$)	p value	PICSI ($n = 117$)	MACS ($n = 110$)	p value
Male age (years)	34.5 ± 6.5	33.1 ± 7.2	0.18	38.0 ± 5.8	38.8 ± 7.2	0.32
SDF (% ± SD)	28.0 ± 7.3	27.8 ± 6.2	0.87	28.7 ± 6.9	29.1 ± 7.8	0.70
MII (%)	81.8 ± 13.7	79.1 ± 13.9	0.29	83.3 ± 13.2	83.8 ± 11.3	0.76
Primary outcome						
Ongoing pregnancy rate (%)*	51.3%	69.5%	0.01	58.8%	47.8%	0.09
Secondary outcomes						
Cleavage rate (% ± SD)	77.0 ± 14.4	75.2 ± 15.8	0.43	74.7 ± 19.1	74.4 ± 18.6	0.88
Blastulation rate (% ± SD)	63.4 ± 18.7	63.7 ± 19.9	0.91	64.9 ± 17.9	63.1 ± 17.9	0.44
Good-quality blastulation rate (% ± SD)	62.9 ± 24.7	71.1 ± 25.4	0.03	64.2 ± 24.3	62.8 ± 31.6	0.69
Clinical pregnancy rate (%)*	58.3%	73.9%	0.03	64.7%	53.2%	0.07
Implantation rate (%)*	37.3%	48.7%	0.1	46%	33.3%	0.05

Data are mean (SD) unless otherwise stated. *n* refers to the number of patients for whom data were available. *PICSI* physiological intracytoplasmic sperm injection, *MACS* magnetic-activated cell sorting. *p* values < 0.05 are considered significant

*Data are percentages only

Discussion

Identifying the paternal factors affecting infertility is critically important for assisted reproduction because ICSI has allowed men with abnormal SDF to successfully father children. Hence, the male age may increase the risk of vertical transmission of paternal genetic defects [4]. Sperm selection in ICSI depends on viability, morphology, and gradient centrifugation; however, the main aim is to select sperm with lower SDF to eliminate the detrimental effects of abnormal SDF [4, 9, 10]. The selection of spermatozoa is crucial in optimizing ICSI outcomes and various techniques are recommended for sperm selection in these patients. Among these techniques are PICSI or MACS that are thought to select rather normal SDF sperm for ICSI [38].

The value of using an advanced sperm selection technique in ICSI has been studied by many researchers. Some supported the usage of those techniques for indicated cases and reported significant improvement in ICSI outcomes, while others who studied their use in general ICSI patients and did not show significant improvements. Ferreyra et al. [26] compared the normal SDF group with the abnormal SDF semen that was processed by MACS; they have found no significant difference between both groups in fertilization, cleavage, blastulation, pregnancy, implantation, and miscarriage rates. They concluded that MACS can eliminate the negative effects of abnormal SDF. Dirican et al. [23] reported significantly higher cleavage and pregnancy rates after using MACS in men with oligoasthenoteratozoospermia. A study by Worrilow et al. [32] used PICSI dishes for sperm selection in 802 patients and found a significant improvement in pregnancy loss rate in patients with hyaluronan binding less than 65% (cutoff value for using PICSI dishes). Also, Majumdar et al. [31] reported an improvement, though not reaching significance, in the miscarriage rate after using PICSI. Contrary to the previous researchers, Horta et al. [27] studied the effect of using MACS on pregnancy, implantation, and miscarriage rates, and did not find any significant improvement in normozoospermic or male factor patients; however, SDF assessment was not carried out. Recently, Miller et al. [39] studied the effect of PICSI on the live birth rate in a parallel two-group prospective randomized trial; the study included all males undergoing ICSI and found that PICSI did not increase the live birth rate compared with ICSI. It is worth noting that those who found improvement in clinical outcomes have applied sperm selection techniques only to patients with abnormal SDF, while those who applied sperm selection as a routine procedure in ICSI have not found an improvement in their clinical outcomes. The study by Miller et al. [39] showed that 72% of the cases selected for PICSI already had a hyaluronan binding score greater than 65% (i.e., not indicated for PICSI). Hence, it seems like the patient's indication for a sperm selection technique is the key to improve their clinical outcomes.

The purpose of our study was to look at which of the two sperm selection techniques (PICSI/MACS) will lead to better clinical outcomes in patients with abnormal SDF. We found that there are no significant differences between PICSI and MACS in all of the studied parameters. Sub-analysis according to the male age showed no significant differences between PICSI and MACS in the same male age group, or even by comparing the 3 different age groups using the same technique (Table 3). This means that either PICSI or MACS can be performed with the same efficiency at different male ages. However, sub-analysis according to the female age showed

that the MACS group had significantly higher good-quality blastulation, clinical pregnancy, and ongoing pregnancy rates than the PICSI group when the female age was less than 30 years (Table 4). Patients allocated to the PICSI group showed a tendency of higher implantation, pregnancy, and ongoing pregnancy rates when the female age is between 30 and 35 years. On the other hand, MACS showed significantly higher good-quality blastulation, implantation, clinical pregnancy, and ongoing pregnancy rates in the female age group less than 30 compared with the older female age group in MACS, while PICSI did not show a significant difference in the outcomes in both female age groups (Table 4).

The process of fertilization in ICSI includes the following steps: oocyte activation, sperm chromatin de-condensation, sperm Aster formation, syngamy, and ends with mitosis [40]. Sperm chromatin de-condensation plays a vital part in this cascade of events [41]. The process of chromatin de-condensation is very much affected by the oocyte's helping mechanisms [42], which are dependent on oocyte's grades of maturity and quality, and are both affected by the maternal age [35, 36]. The first step in chromatin de-condensation, reorganization, and activation of paternal genome is mediated by an enzyme (splicing kinase SRPK1), which only present in the oocyte [43]; it also plays a role in synchronizing the remodeling of paternal genome with that of the maternal genome [43]. PICSI and MACS have different mechanisms for sperm selection [38]. MACS-selected sperm display lower SDF, decreased apoptosis, and higher early fertilization potential than conventional ICSI [44, 21, 24, 27]. This can be explained by early sperm chromatin de-condensation [44]. On the other hand, PICSI does not select sperm according to apoptosis, but rather upon their maturity and hyaluronan binding [45]. It does appear that the advantage of MACS in sperm selection becomes much clearer depending on the female age as a proxy of oocyte quality [35]. Studies that have looked at oocyte quality reflected by meiotic errors and the genesis of human aneuploidy showed that the oocyte quality is better at younger ages below 30 in comparison with age beyond 30 [46]. Therefore, this supports our finding that MACS-selected sperm result in better pre-implantation embryogenesis than PICSI-selected sperm when the female age is less than 30 years.

The efficiency of sperm selection techniques is still debatable and there are not enough clinical trials comparing different techniques. Screening the literature retrospectively, only one study by Troya et al. [47] compared two different sperm selection techniques, where they compared PICSI and MACS and found that MACS had significantly higher fertilization and clinical pregnancy rates. When the female age is less than 30 years, our results are consistent with the study by Troya and coworkers [47], where the mean female age was 36.9 years. There are differences between both studies where we included a higher number of patients (396 patients), which is statistically powered to show the difference, while Troya et al. included only 136 patients. Moreover, their main endpoint was the clinical pregnancy rate, while we reported the implantation rate and extended the evaluation of pregnancy to the ongoing pregnancy rate, since abnormal SDF may cause later problems like miscarriage [12]. We used a direct method (TUNEL) for the evaluation of SDF instead of their indirect method (Sperm chromatin dispersion) [48]. In addition, they did not measure the SDF of patients before randomization.

While this study has several strengths such as clearly defined outcomes, statistically powered to show the difference, and it is the largest registered clinical trial comparing two different sperm selection techniques, on the other hand, the limitations of the study include no control since it was very difficult to recruit patients with abnormal SDF. Another advantage would have been if the SDF could have been performed on the same ejaculates that have been used for injection. In addition, it would be better if the follow-up of the outcomes was extended to the live birth rate, and this will be considered in our future research plans.

Conclusion

MACS and PICSI are suitable and efficient tools for human sperm selection in patients undergoing ICSI with abnormal SDF. Both techniques showed approximately similar results in the primary analysis. MACS can achieve higher good-quality blastulation, clinical pregnancy, and ongoing pregnancy rates in females age less than 30, while PICSI is trending to have higher implantation, clinical pregnancy, and ongoing pregnancy rate in female age between 30 and 35.

Compliance with ethical standards

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

Ethical approval This study was reviewed, discussed, and approved by Ganin Fertility Center ethics committee in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The committee approved the study before starting it and assures that the research plans are reasonable and participants are adequately protected.

Consent to participate Informed consent was obtained from all individual participants included in the study before their inclusion.

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