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ORIGINAL ARTICLE

Male Infertility

Use of testicular sperm in couples with SCSA-defined high sperm DNA fragmentation and failed intracytoplasmic sperm injection using ejaculated sperm

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Sperm DNA fragmentation (SDF) has been linked with male infertility, and previous studies suggest that SDF can have negative influence on pregnancy outcomes with assisted reproduction. We performed a retrospective review of consecutive couples with a high SDF level that had intracytoplasmic sperm injection (ICSI) using testicular sperm (T-ICSI). We compared the T-ICSI outcomes to that of two control groups: 87 couples with failed first ICSI cycle and who had a second ICSI cycle using ejaculated sperm (Ej-ICSI), and 48 consecutive couples with high sperm chromatin structure assay (SCSA)-defined SDF (>15%) that underwent an ICSI cycle using ejaculated sperm after one or more failed ICSI cycles (Ej-ICSI-high SDF). The mean number of oocytes that were retrieved and the total number of embryos were not different among the three groups. The mean number of transferred embryos in the T-ICSI group was higher than the Ej-ICSI group but not significantly different than the Ej-ICSI-high SDF group (1.4, 1.2, and 1.3, respectively, $P < 0.05$). Clinical pregnancy rate in the T-ICSI group was not significantly different than the Ej-ICSI and Ej-ICSI-high SDF groups (48.6%, 48.2%, and 38.7%, respectively, $P > 0.05$). No significant difference was found in live birth rate when comparing T-ICSI to Ej-ICSI and Ej-ICSI-high SDF groups. The results suggest that pregnancy outcomes and live birth rates with T-ICSI are not significantly superior to Ej-ICSI in patients with an elevated SCSA-defined sperm DNA fragmentation and prior ICSI failure(s).

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INTRODUCTION

Fifteen percent of couples are affected by infertility, rendering nearly one of six couples unable to produce offspring.[1] A male factor is the reason for infertility in up to 50.0% of couples. Investigators have demonstrated that fragmentation or disturbances in sperm DNA integrity in male factor infertility may lead to aberrations in embryo development, fertilization, and implantation. Currently, there is good evidence that infertile men have substantially more sperm DNA damage compared to fertile men[2–8] and that this DNA damage may negatively impact natural reproduction, intrauterine insemination (IUI)-assisted reproduction, and *in vitro* fertilization (IVF) pregnancy.[9–11] Moreover, in a meta-analysis and systematic review by Zini *et al.*[12] in 2008, they have shown that sperm DNA damage is linked to an increased risk of pregnancy loss after IVF and intracytoplasmic sperm injection (ICSI).

Several studies have proposed that the source of sperm used for IVF/ICSI may impact fertility and pregnancy outcome with ICSI. Greco *et al.*[13] in 2005 described a higher pregnancy rate with ICSI upon using testicular compared to ejaculated sperm from infertile patients with an elevated level of sperm DNA damage. More recently, Esteves *et al.*[14] conducted a meta-analysis of available studies on testicular sperm-ICSI in nonazoospermic couples with an elevated sperm DNA fragmentation (SDF) level. These investigators reported that lower levels of SDF were found in testicular compared to ejaculated sperm, and ICSI using testicular sperm (T-ICSI) was associated with higher pregnancy rates and lower miscarriage rates than using ejaculated sperm (Ej-ICSI). A recent study by Arafa *et al.*[15] demonstrated that in a cohort of infertile men with high SDF, the clinical pregnancy rate is significantly better when testicular rather than ejaculated sperm is used for ICSI.

The higher DNA damage that observed in ejaculated compared to testicular sperm suggests that sperm DNA injury may be acquired during epididymal transit or ejaculation. This is supported by experimental animal studies showing that the sperm passage through the epididymis was linked to the loss of sperm DNA integrity and capacity to fertilize.[16]

The aim of the current study was to further explore the pregnancy outcomes using T-ICSI in nonazoospermic couples who have failed ICSI cycles using Ej-ICSI.

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PATIENTS AND METHODS

Patients

We performed a retrospective review of 52 nonazoospermic couples with moderately high SDF and high SDF (>15.0% SDF and >30.0% SDF, respectively, by sperm chromatin structure assay [SCSA]) that had T-ICSI after one or more failed ICSI cycle(s) using ejaculated sperm from December 2012 to July 2017 at the OVO Fertility Clinic in Montreal, Canada. We compared the T-ICSI outcomes to that of two cohorts that were treated at the same institution, during the same period: 87 consecutive couples with failed first ICSI cycle and had a second ICSI cycle using ejaculated sperm (Ej-ICSI), and 48 consecutive couples with moderately high SDF and high SDF (>15.0% SDF and >30.0% SDF, respectively, by SCSA) that underwent an ICSI cycle using ejaculated sperm after one or more failed ICSI cycles (Ej-ICSI-high SDF). We excluded couples with advanced female age (>40 years) and female factors. We also excluded couples with a correctable male factor (*e.g.*, varicocele and semen infection).

Written consent for the testicular aspiration was obtained from patients with a clear understanding of the risks associated with testicular sperm retrieval. There was no written consent for the use of testicular versus ejaculated sperm in the setting of failed ICSI and high DFI. However, there was a thorough discussion about the pros and cons of testicular sperm aspiration (TESA) in this setting. Patients were made aware of the experimental nature of the procedure (T-ICSI in context of high DFI). Patients were made aware of the positive but limited data favoring TESA in this setting and were able to make an informed decision on how they wished to proceed. The research and development scientific committee at OVO clinic reviewed our study, and we acquired the approval as a quality control study. We followed the Helsinki Declaration principle.

Semen analysis was done using a microptic SCA (Sperm Class Analyzer, Microptic, Barcelona, Spain) with measurements of sperm motility taken at 37°C. The assessment of sperm DNA damage was performed by the SCSA, and we expressed the results as percent sperm DNA fragmentation index (%DFI, an index of DNA damage), as previously described.[6,9,17] Briefly, stored semen samples were thawed on ice and treated for 30 s with acid solution and 3 min with acridine orange.[6,7] A minimum of 5000 cells from two aliquots of each sample were analyzed by FACS scan interfaced with a data handler (CellQuest 3.1; Becton Dickinson, Franklin Lakes, NJ, USA) on a Power Macintosh 7600/132 computer (Cupertino, CA, USA). WinList (Verity Softwarehouse Inc., Topsham, ME, USA) was utilized to generate the cytogram, as well as, %DFI readings. A mean %DFI value from two sperm samples was reported.

All men were evaluated in our clinic with a thorough history, physical examination, and relevant laboratory testing. At our IVF center, TESA is done fresh the day before oocyte retrieval in keeping with our embryologist's preference. Before performing TESA-ICSI, every case was first reviewed by the clinical team (urologist, gynecologist, and embryologist). Couples who failed the first ICSI cycles using ejaculate sperm and with moderately high SDF and high SDF (>15.0% SDF and >30.0% SDF, respectively, by SCSA) were offered T-ICSI as an alternative to Ej-ICSI. The experimental nature of T-ICSI was discussed as was the potential benefits and risks of testicular sperm retrieval (bleeding, infection, pain, hypogonadism, and unknown genetic and epigenetic risks).

For ovarian stimulation, we used a gonadotropin-releasing hormone (GnRH) antagonist protocols utilizing either recombinant follicle-stimulating hormone (FSH) or human menopausal gonadotropin (HMG) depending on physician's choice with dose based on patients' ovarian reserve markers on day 3 of menstrual cycle, antral follicle count (AFC), anti-Müllerian hormone (AMH), and FSH. When the leading follicle reached 14 mm in size, all patients received a GnRH antagonist (Ganirelix, Orgalutran; Organon, Ontario, Canada) daily until the day of human chorionic gonadotropin (HCG) injection. Once the follicular size reached 17 mm or greater, HCG (Pregnyl, Organon) was administered subcutaneously. Oocyte retrieval took place 34 h later. On the day of retrieval, endovaginal sonography was performed before collection to confirm the presence of a follicle.

We collected the following variables in our study: patient age, partner age, female body mass index, type of infertility, serum FSH level, total testosterone, testicular volume, semen volume, sperm concentration, sperm total motility, sperm normal morphology, sperm %DFI for the T-ICSI group, sperm progressive motility, number of oocytes and metaphase II oocytes retrieved, fertilization rate (defined as the number of 2 pronuclei - 2PNs - divided by the number of metaphase II oocytes), total number of embryos, total number of embryos transferred (ET), number of cycles with failed embryo development, clinical pregnancy rate (per embryo transfer), miscarriage rate (pregnancy loss after clinical pregnancy), and live birth rate (per embryo transfer). We included only fresh embryo transfers in our analysis. We established the clinical pregnancy by detecting the fetal heartbeat on ultrasound after 8 weeks of gestation. The miscarriage rate was calculated as clinical pregnancies that did not result in a live birth per clinical pregnancy. Live birth rate was calculated as the ratio between the number of deliveries resulting in at least one live birth and the number of ETs. For live birth data, we excluded couples who had missing information about birth or had an abortion. We also performed a subgroup analysis for ICSI outcomes in T-ICSI group and Ej-ICSI-high SDF group for patients with %DFI <30.0% and ≥30.0%.

Testicular sperm retrieval

Testicular sperm retrieval was performed by TESA under local anesthesia and all procedures were performed by the same surgeon (AZ), as previously described.[18] Briefly, after administration of local anesthesia, a 16-gauge clear angiocatheter needle (1-1/4" Cathlon IV Catheter, Smiths Medical International Ltd., Rossendale, UK) is directed through the scrotal skin into the testis. The needle is withdrawn and the angiocatheter is kept in place. A 10 ml syringe containing approximately 2 ml of sperm buffer is attached to the angiocatheter. Negative pressure is created and the angiocatheter is gently withdrawn and then pushed back into testis until testicular tissue appears in the syringe. At this point, the angiocatheter is withdrawn completely while maintaining negative pressure. The aspirated testicular tissue is expelled into a sterile dish. The specimen is immediately dissected and then examined under the microscope to confirm the presence of spermatozoa. In all of the TESA procedures, large numbers of motile spermatozoa were recovered in numbers sufficient for subsequent ICSI.

Statistical analyses

IBM Statistical Package for the Social Sciences (SPSS, version 20; SPSS Inc., IBM Corp., Armonk, NY, USA) was used to collect data and perform statistical analysis. Continuous variables were compared using the Mann–Whitney U test. Chi-square test was used to compare dichotomous variables. A $P < 0.05$ was considered statistically significant.

RESULTS

We identified 52 couples with one or more failed Ej-ICSI with moderately high and high SDF that underwent T-ICSI. The mean

%DFI of the couples in the TESA group was 37.6%, with 70.0% having a %DFI >30.0%. Thirty-seven couples in the T-ICSI group underwent a fresh ET (4 of these couples had no embryo development and 11 had immediate cryopreservation of embryos for later ET). For live birth data, we excluded four couples with three missing information about birth and one who had an abortion.

All men in the T-ICSI cohort underwent a unilateral TESA with a 100% sperm retrieval rate. None of the patients required a bilateral TESA. Although no early complications were reported, we could not sufficiently assess the TESA complication rate because the majority of the patients did not return for follow-up after TESA.

We evaluated the outcomes of 87 consecutive couples who underwent a second ICSI cycle with ejaculated sperm after failed first ICSI cycle (Ej-ICSI) and 48 consecutive couples with moderately high and high SDF and one or more failed ICSI cycles that underwent an ICSI cycle using ejaculated sperm (Ej-ICSI-high SDF). Fifty-six couples in the Ej-ICSI group underwent a fresh ET (8 had no embryo development and 23 had immediate cryopreservation of embryos for later ET) and 31 couples in the Ej-ICSI-high SDF group underwent a fresh ET (7 had no embryo development and 10 had immediate cryopreservation of embryos for later ET). For live birth data, we excluded eight couples in Ej-ICSI group and one couple in Ej-ICSI-high SDF group because of missing information about birth.

The baseline characteristics of the T-ICSI, Ej-ICSI, and Ej-ICSI-high SDF groups are shown in **Table 1** and **2.** The mean male and female ages, the proportion of couples with primary infertility, the number of retrieved oocytes, the mean number of embryos, and the proportion of cycles with failed embryo development were not significantly different between the T-ICSI and the Ej-ICSI and Ej-ICSI-high SDF groups (all $P > 0.05$). However, the percent progressive motility and fertilization rate in the T-ICSI group were both significantly lower than those in the Ej-ICSI and Ej-ICSI-high SDF groups (both $P < 0.05$). Total testosterone, sperm concentration, and total motility were significantly higher in Ej-ICSI-high SDF group compared to those in T-ICSI group (all $P < 0.05$). Mean sperm %DFI was significantly higher in T-ICSI compared to that in the Ej-ICSI-high SDF group ($P < 0.05$), whereas the mean number of prior ICSI cycles was significantly higher in Ej-ICSI-high SDF compared to that in the T-ICSI group ($P < 0.05$).

The pregnancy outcomes of the T-ICSI, Ej-ICSI, and Ej-ICSI-high SDF groups are shown in **Table 3** and **4**. The mean number of transferred embryos was significantly higher in T-ICSI compared to that in the Ej-ICSI group (**Table 3**, $P < 0.05$). However, clinical pregnancy rate per fresh ET, miscarriage rate, and live birth rate in the T-ICSI group were not significantly different than those in the Ej-ICSI and Ej-ICSI-high SDF groups (**Table 3** and **4**, all $P > 0.05$). Subgroup analysis based on %DFI cutoff (<30.0% and ≥30.0%) was performed to compare the ICSI outcomes between T-ICSI and Ej-ICSI-high SDF groups and it did not reveal any significant differences in outcomes (**Table 5**).

DISCUSSION

We have conducted a retrospective study on the reproductive outcomes of a cohort of nonazoospermic couples with high %DFI and prior ICSI failure(s) undergoing T-ICSI. We have demonstrated that T-ICSI is associated with a favorable clinical pregnancy rate per embryo transfer (48.6% clinical pregnancy rate per transfer with a mean of 1.4 embryos transferred). These couples had a mean of two prior ICSI failures and a mean %DFI of 37.6%. All men in the T-ICSI cohort underwent a unilateral TESA with a 100% sperm retrieval rate.

Table 1: Characteristics of testicular (intracytoplasmic sperm injection using testicular sperm) group and ejaculate (intracytoplasmic sperm injection cycle using ejaculated sperm) group (first control)

Parameter	*T-ICSI (n=52)*	*Ej-ICSI (control, n=87)*	*P*
Age (year), mean±s.d.			
Male	38.9±5.9	37.0±6.6	0.051
Female	34.4±3.7	33.5±4.8	0.3
Female body mass index (kg m^{-2}), mean±s.d.	23.0±3.4	24.0±4.8	0.87
Type of infertility, *n* (%)			
Primary	36 (69.2)	62 (71.3)	0.8
Secondary	16 (30.8)	25 (28.7)	
Serum FSH level (IU l^{-1}), mean±s.d.	6.8±7.2	7.5±7.7	0.85
Total testosterone (nmol l^{-1}), mean±s.d.	12.0±4.0	13.0±5.4	0.24
Right testicular volume (ml), mean±s.d.	18.0±2.7	17.0±3.6	0.13
Left testicular volume (ml), mean±s.d.	17.0±2.4	16.0±3.6	0.11
Spermogram, mean±s.d.			
Volume (ml)	3.1±1.7	3.0±1.5	0.55
Concentration ($\times 10^6$ ml^{-1})	22.9±31.6	35.0±47.0	0.09
Total motility (%)	42.0±23.9	48.3±20.9	0.06
Normal morphology (%)	1.9±1.7	2.3±1.5	0.13
DFI (%)	37.6±15.9	NA	NA
A+B	0.27±0.20	0.45±0.26	<0.001
Number of oocytes, mean±s.d.			
Retrieved	11.2±5.6	12.6±5.9	0.17
MII	10.3±5.3	10.1±5.0	0.74
Fertilization rate, mean±s.d.	0.58±0.27	0.72±0.21	0.004
Cycles with failed embryo development, *n* (%)	4/52 (7.7)	8/87 (9.2)	1.0
Number of total embryos, mean±s.d.	6.2±4.1	7.0±3.6	0.51

DFI: DNA fragmentation index; MII: metaphase II; FSH: follicle-stimulating hormone; ICSI: intracytoplasmic sperm injection; T-ICSI: ICSI using testicular sperm; Ej-ICSI: ICSI cycle using ejaculated sperm; s.d.: standard deviation; A+B: progressive motility; NA: not available

Table 2: Characteristics of testicular (intracytoplasmic sperm injection using testicular sperm) group and ejaculate (intracytoplasmic sperm injection cycle using ejaculated sperm-high sperm DNA fragmentation) group (second control)

Parameter	*T-ICSI (n=52)*	*Ej-ICSI-high SDF (control, n=48)*	*P*
Age (year), mean±s.d.			
Male	38.9±5.9	39.7±6.5	0.54
Female	34.4±3.7	35.5±3.3	0.2
Female body mass index (kg m^{-2}), mean±s.d.	23.0±3.4	24.0±4.7	0.7
Type of infertility, *n* (%)			
Primary	36 (69.2)	31 (64.6)	0.62
Secondary	16 (30.8)	17 (35.4)	
Serum FSH level (IU l^{-1}), mean±s.d.	6.8±7.2	4.6±1.8	0.3
Total testosterone (nmol l^{-1}), mean±s.d.	12.0±4.0	16.0±7.1	0.01
Right testicular volume (ml), mean±s.d.	18.0±2.7	19.0±3.1	0.22
Left testicular volume (ml), mean±s.d.	17.0±2.4	18.0±3.0	0.4
Spermogram, mean±s.d.			
Volume (ml)	3.1±1.7	2.7±1.1	0.15
Concentration ($\times 10^6$ ml^{-1})	22.9±31.6	41.2±49.9	0.006
Total motility (%)	42±23.9	52±22.8	0.03
Normal morphology (%)	1.9±1.7	1.8±1.6	0.9
DFI (%)	37.6±15.9	26±8.6	<0.001
A+B	0.27±0.2	0.49±0.22	<0.001
Number of oocytes, mean±s.d.			
Retrieved	11.2±5.6	11.5±6.7	0.87
MII	10.3±5.3	9.0±6.0	0.1
Fertilization rate, mean±s.d.	0.58±0.27	0.7±0.23	0.03
Prior ICSI, mean±s.d.	2.0±1.0	3.3±1.3	<0.001
Cycles with failed embryo development, *n* (%)	4/52 (7.7)	7/48 (14.6)	0.27
Total embryos, mean±s.d.	6.2±4.1	5.4±4.2	0.29

DFI: DNA fragmentation index; MII: metaphase II; FSH: follicle-stimulating hormone; ICSI: intracytoplasmic sperm injection; T-ICSI: ICSI using testicular sperm; Ej-ICSI: ICSI cycle using ejaculated sperm; s.d.: standard deviation; SDF: sperm DNA fragmentation; A+B: progressive motility

Table 3: Intracytoplasmic sperm injection outcomes in testicular (intracytoplasmic sperm injection using testicular sperm) group and ejaculate (intracytoplasmic sperm injection cycle using ejaculated sperm) group (first control)

Parameter	*T-ICSI (n=37)*	*Ej-ICSI (n=56)*	*P*
Number of embryo transfer, mean±s.d.	1.4±0.5	1.2±0.3	0.008
CPR per ET, *n* (%)	18/37 (48.6)	27/56 (48.2)	1.0
Miscarriage rate, *n* (%)	2/18 (11.1)	3/27 (11.1)	1.0
Live birth rate, *n* (%)	12/33 (36.4)[a]	16/48 (33.3)[b]	0.77

[a]No information about birth in 3 couples, 1 couple had an abortion. [b]No information about birth in 8 couples. CPR: clinical pregnancy rate; ET: embryo transfer; ICSI: intracytoplasmic sperm injection; T-ICSI: ICSI using testicular sperm; Ej-ICSI: ICSI cycle using ejaculated sperm; s.d.: standard deviation

Table 4: Intracytoplasmic sperm injection outcomes in testicular (intracytoplasmic sperm injection using testicular sperm) group and ejaculate (intracytoplasmic sperm injection cycle using ejaculated sperm intracytoplasmic sperm injection cycle using ejaculated sperm-high sperm DNA fragmentation) group (second control)

Parameter	*T-ICSI (n=37)*	*Ej-ICSI-high SDF (n=31)*	*P*
Number of embryo transfer, mean±s.d.	1.4±0.5	1.3±0.5	0.41
CPR per ET, *n* (%)	18/37 (48.6)	12/31 (38.7)	0.41
Miscarriage rate, *n* (%)	2/18 (11.1)	2/12 (16.7)	0.66
Live birth rate, *n* (%)	12/33 (36.4)[a]	9/30 (30.0)[b]	0.59

[a]No information about birth in 3 couples, 1 couple had an abortion. [b]No information about birth in 1 couple. CPR: clinical pregnancy rate; ET: embryo transfer; ICSI: intracytoplasmic sperm injection; T-ICSI: ICSI using testicular sperm; Ej-ICSI: ICSI cycle using ejaculated sperm; s.d.: standard deviation; SDF: sperm DNA fragmentation

To date, there is no consensus in the literature as to what threshold should be used as the cutoff to select couples for testicular rather than ejaculated sperm for ICSI.[19] We selected the 15% cutoff based on a study by Evenson *et al.*[9] demonstrating that couples with a %DFI >15.0% had poor reproductive outcomes compared to couples with a %DFI <15.0%. This is also the same cutoff (%DFI >15.0%) that was utilized by Greco *et al.*[13] We categorized the patients as having moderately high %DFI (%DFI >15.0% and <30.0%) and high %DFI (>30.0%).

We compared the pregnancy outcomes of T-ICSI to those of a cohort from the same institution with a prior ICSI failure but without assessment of sperm DNA fragmentation. We identified 87 consecutive couples that failed a first ICSI cycle and underwent a second Ej-ICSI. Although this cohort did not have sperm DNA fragmentation testing, this group was felt to be an adequate comparison group to evaluate differences in clinical pregnancy rates because sperm %DFI has been shown to have no or possibly minimal influence on ICSI pregnancy rates.[10,11] Our data show that the clinical pregnancy rate per fresh ET, miscarriage rate, and live birth rate in the T-ICSI group were not significantly different than those in the Ej-ICSI group (48.6% *vs* 48.2%, 11.1% *vs* 11.1%, and 36.4% *vs* 33.3%, respectively, all $P > 0.05$). However, it is important to note that these two groups were not perfectly matched. Indeed, the mean number of transferred embryos was significantly higher in T-ICSI compared to the Ej-ICSI group which would favor the T-ICSI cohort pregnancy rate ($P < 0.05$).

We also compared the pregnancy outcomes of T-ICSI to those of a cohort from the same institution with prior ICSI failure(s) and high %DFI. We identified 48 consecutive couples who failed one or more ICSI cycles and underwent a subsequent ICSI cycle using ejaculated sperm (Ej-ICSI-high SDF). Our data show that clinical pregnancy rate per fresh ET, miscarriage rate, and live birth rate in the T-ICSI group were not significantly different than those in the Ej-ICSI-high SDF

Table 5: Subgroup analysis for intracytoplasmic sperm injection outcomes in testicular (intracytoplasmic sperm injection using testicular sperm) group and ejaculate (intracytoplasmic sperm injection cycle using ejaculated sperm-high sperm DNA fragmentation) group (second control) in patients with (percent sperm DNA fragmentation index <30%) and (percent sperm DNA fragmentation index ≥30%)

Parameter	*%DFI <30%*			*%DFI ≥30%*		
	T-ICSI (n=12)	*Ej-ICSI-high SDF (n=23)*	*P*	*T-ICSI (n=25)*	*Ej-ICSI-high SDF (n=8)*	*P*
CPR per ET, *n* (%)	6/12 (50.0)	10/23 (43.5)	0.7	12/25 (48.0)	2/8 (25.0)	0.25
Miscarriage rate, *n* (%)	0/6 (0)	2/10 (20.0)	0.24	2/12 (16.7)	0/2 (0)	0.53
Live birth rate, *n* (%)	5/9 (55.5)	7/22 (31.8)	0.21	7/24 (29.2)	2/8 (25.0)	0.82

CPR: clinical pregnancy rate; ET: embryo transfer; ICSI: intracytoplasmic sperm injection; T-ICSI: ICSI using testicular sperm; Ej-ICSI: ICSI cycle using ejaculated sperm; SDF: sperm DNA fragmentation; %DFI: percent sperm DNA fragmentation index

group (48.6.0% *vs* 38.7%, 11.1% *vs* 16.7%, 36.4% *vs* 30.0%, respectively, all $P > 0.05$). This cohort was felt to be an adequate comparison group to evaluate differences in clinical pregnancy and miscarriage rates between T-ICSI and Ej-ICSI groups; however, it is important to note that these two groups were not perfectly matched. The mean sperm %DFI was significantly higher in T-ICSI than that in the Ej-ICSI-high SDF group, whereas the mean number of prior ICSI cycles was significantly higher in Ej-ICSI-high SDF than that in the T-ICSI group. The couples undergoing Ej-ICSI had a higher sperm motility than the couples undergoing TESA-ICSI strongly suggesting that the former had a lower mean %DFI than the TESA cohort because, as we and others have shown, sperm %DFI is inversely correlated to sperm motility.[20,21] Moreover, we observed that there was a trend toward poorer embryo development in the Ej-ICSI group compared to the T-ICSI group. We speculate that the poorer embryo development in the Ej-ICSI group may be related to the "late paternal effect" phenomenon which is described as an abnormality in the paternal sperm genome integrity that affects postfertilization embryo development.[22]

The rationale for using testicular rather than ejaculated sperm for ICSI in couples with sperm DNA fragmentation is based on studies, demonstrating marginally poorer pregnancy outcomes in couples with DNA damage, and the observation that sperm DNA fragmentation is significantly lower in testicular compared to ejaculated sperm.[11,13] Indeed, in 2005, Greco *et al.*[13] described higher pregnancy rates with T-ICSI compared to Ej-ICSI in couples with sperm DNA damage and observed a higher frequency of sperm exhibiting detectable DNA damage in ejaculated compared to testicular sperm. These observations were supported by experimental studies demonstrating that in animals with spermatogenesis abnormalities, the sperm passage through the epididymis was linked to the loss of sperm DNA integrity and capacity to fertilize.[16] Subsequent studies have similarly shown that sperm DNA fragmentation is significantly lower in testicular compared to ejaculated sperm in men with sperm DNA damage.[23,24]

Several studies have demonstrated that T-ICSI is associated with higher pregnancy rates compared to Ej-ICSI in couples with SDF (with or without prior ICSI failure).[13,15,25,26] These findings have led clinicians to utilize testicular rather than ejaculated sperm for ICSI in men with spermatogenesis abnormalities and poor sperm DNA integrity. In an online survey of Canadian fertility clinics (in 2015), 70.0% of the respondents reported performing T-ICSI for patients with sperm DNA fragmentation (Zini *et al*., unpublished observations). Similarly, over 70.0% of the respondents attending a session on testicular sperm for ICSI at the 2017 Annual Meeting of the American Society for Reproductive Medicine reported that they would (in selected cases) opt for testicular rather than ejaculated sperm ICSI in couples with sperm DNA damage (Zini *et al*., unpublished observations). However, the available studies on T-ICSI in couples with SDF are small and largely retrospective studies with variable sperm DNA assays and assay thresholds. As such, there is no consensus or guideline on how to manage these cases in clinical practice.

This is the first study on T-ICSI for couples with SDF showing no superiority of T-ICSI over Ej-ICSI. Although our study is retrospective, the number of cycles we evaluated would be sufficient to detect a significant difference in CPR between groups assuming clinical pregnancy rates in the T-ICSI and Ej-ICSI groups of 45.0% and 20.0%, respectively.[13,15,23,26] An important limitation of our study is that the T-ICSI and Ej-ICSI groups were not perfectly matched. Clearly, these data indicate that there is a demand for randomized controlled trials to determine the value of T-ICSI in couples with sperm DNA damage.

AUTHOR CONTRIBUTIONS

MA, AZ, SP, and JIK designed the study. MA and AZ analyzed the data. MA, AZ, and FH wrote the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

AZ and JIK are shareholders in Yad-Tech Nutraceutical. Other authors declare no competing interests.

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