Comparison of reproductive outcome in oligozoospermic men with high sperm DNA fragmentation undergoing intracytoplasmic sperm injection with ejaculated and testicular sperm

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Objective: To investigate the effectiveness of intracytoplasmic sperm injection (ICSI) using testicular sperm as a strategy to overcome infertility in men with high sperm DNA fragmentation (SDF).

Design: Prospective, observational, cohort study.

Setting: Private IVF centers.

Patient(s): A total of 147 couples undergoing IVF-ICSI and day 3 fresh ETs whose male partner has oligozoospermia and high SDF.

Intervention(s): Sperm injections were carried out with ejaculated sperm (EJA-ICSI) or testicular sperm (TESTI-ICSI) retrieved by either testicular sperm extraction (TESE) or testicular sperm aspiration (TESA). SDF levels were reassessed on the day of oocyte retrieval in both ejaculated and testicular specimens.

Main Outcome Measure(s): Percentage of testicular and ejaculated spermatozoa containing fragmented DNA (%DFI) and clinical pregnancy, miscarriage, and live-birth rates.

Result(s): The %DFI in testicular sperm was 8.3%, compared with 40.7% in ejaculated sperm. For the TESTI-ICSI group versus the EJA-ICSI group, respectively, the clinical pregnancy rate was 51.9% and 40.2%, the miscarriage rate was 10.0% and 34.3%, and the live-birth rate was 46.7% and 26.4%.

Conclusion(s): ICSI outcomes were significantly better in the group of men who had testicular sperm used for ICSI compared with those with ejaculated sperm. SDF was significantly lower in testicular specimens compared with ejaculated counterparts. Our results suggest that TESTI-ICSI is an effective option to overcome infertility when applied to selected men with oligozoospermia and high ejaculated SDF levels. (Fertil Steril 2015; –: ––

Key Words: Ejaculation, infertility, male/therapy, intracytoplasmic sperm injection, sperm DNA fragmentation, sperm retrieval

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may be clinically informative for IUI or IVF and intracytoplasmic sperm injection (ICSI) outcomes (5).

A few noninvasive strategies have been proposed to alleviate SDF and/or select sperm with higher quality chromatin content for IVF and ICSI. The intake of oral antioxidants (6, 7), varicocele repair (8), and the use of recurrent ejaculations before fertilization (9, 10) alone or combined with micromanipulation-based sperm selection techniques such as magnetic cell sorting (11, 12), physiological ICSI (13), or intracytoplasmic morphologically selected sperm injection (14–16) have been attempted with varying success rates to decrease SDF. Yet none of these interventions, alone or combined, have been unequivocally proven to be of clinical value to bypass the potential detrimental effect of abnormal SDF on assisted reproductive technology (ART) outcomes (5).

Spermatozoa retrieved from the testis of men with abnormal ejaculated sperm DNA integrity tend to have better DNA quality (17, 18). However, the synergistic combination of testicular sperm retrieval and ICSI in oligozoospermic men with high ejaculated SDF has been poorly studied. The present study aimed to investigate the effectiveness of ICSI using testicular sperm as a strategy to overcome infertility in men with oligozoospermia and high SDF.

MATERIAL AND METHODS

A prospective, observational, cohort study was conducted between January 2011 and December 2013 in two private IVF centers in Spain and Brazil. The Institutional Review Board of the participating institutions approved the study, and informed consent was obtained from all participants.

Patient Selection

The following inclusion criteria were established: [1] infertility duration > 1 year; [2] women and men ages 39 and 45 years old or less, respectively, undergoing IVF-ICSI; [3] idiopathic oligozoospermia (<15 million/mL), with no obvious abnormalities noted in the medical history, physical examination, and endocrine profile; [4] persistent high SDF levels (>30%) in two semen specimens after oral antioxidant treatment for a minimum of 3 months; and [5] no evidence of subclinical genital infections and/or leukocytospermia. Exclusion criteria included [1] severe male factor infertility (severe oligozoospermia, <5 million/mL; and azoospermia); [2] women with history of poor response to ovarian stimulation, including those fitting the Bologna criteria for expected poor responders (19); [3] patients in whom oocyte or sperm donation was used as part of the treatment strategy; and [4] uterine pathology. Only the first ICSI-ET cycle of each patient using fresh sperm for injections was included in this analysis. Oral antioxidant therapy varied according to the prescribing physicians but included a combination of commercially available vitamins (C, E, and folic acid) and supplements (selenium and zinc).

SDF Analysis

Samples were collected by masturbation after 2–3 days of ejaculatory abstinence. After liquefaction, specimens were assessed for volume, count, motility, vitality, morphology, and leukocytes, in accordance with the fifth edition of the World Health Organization laboratory manual for the examination and processing of human semen (20). SDF was assessed using the sperm chromatin dispersion (SCD) test according to the protocol described by Fernández et al. (21). To avoid iatrogenic DNA damage, sperm specimens were assessed for DNA fragmentation 1 hour after ejaculation.

SDF was reassessed on the day of oocyte pick-up in both neat semen and testicular specimens. A variant of the Halo-sperm test (Halotech DNA) that combines a dual fluorescent cocktail probe to discriminate somatic cells from spermatozoa was used. This technique was described elsewhere to study a patient with Kartagener syndrome and recurrent implantation failure (22). Briefly, GelRed (Biotium) was used for DNA staining, and 2.7-dibrom-4-hydroxy-mercury-fluorescein (Sigma-Aldrich) for protein staining. Images were captured with a black and white cooled camera (Leica DCF 300) mounted onto an epifluorescence microscope (DMRB; Leica Microsystems) equipped with an appropriate single-band filter block for each fluorochrome (Semrock). A minimum of 200 spermatozoa per sample was scored. The spermatozoa with very small or no halos were defined as the cells containing fragmented DNA, and results were expressed as the percentage of sperm with DNA fragmentation (%DFI; DNA fragmentation index).

Testicular Sperm Retrieval

Retrievals were performed by testicular sperm extraction (TESE) or testicular sperm aspiration (TESA), as described elsewhere (23). The choice between the two methods was based purely on surgeon preference. All procedures were carried out under local anesthesia combined with an IV bolus infusion of propofol on an outpatient basis (24). The extracted testicular tissue was flushed into a tube containing sperm medium. Subsequently, the seminiferous tubules were mechanically minced using fine needles attached to tuberculin syringes to ensure wall breakdown and cellular content loss (25, 26). Successful retrieval was defined as the presence of an adequate number of motile sperm for sperm injections.

Study Protocol

Couples were subjected to IVF-ICSI using either ejaculated sperm collected after an abstinence period of 2–3 days (EJA-ICSI) or testicular sperm (TESTI-ICSI) retrieved by TESE or TESA. All sperm injections were performed with fresh specimens. Controlled ovarian stimulation was carried out with recombinant FSH (Gonal-f, Merck Serono), starting on day 2 or 3 after onset of menses, with doses ranging from 112.5 to 300 IU per day according to the patient age, ovarian reserve, and the current practice of attending physicians. A daily administration of GnRH antagonist (cetrorelix 0.25 mg; Cetrotide, Merck Serono) for pituitary suppression was prescribed when the leading follicle achieved 13 mm. Recombinant hCG (rec-hCG 250 μg; Ovidrel; Merck Serono) was administered SC for final oocyte maturation when at least two follicles reached a mean diameter of 17 mm. All patients were subjected to transvaginal ultrasound-guided oocyte pick-up.
36 hours after rec-hCG administration. The fertilized oocytes were cultured, and embryo quality was assessed. Abdominal ultrasound-guided ET on day 3 after oocyte pick-up was carried out, and all patients started luteal phase support with a single daily administration of vaginal P in gel (Crinone 8%, Merck Serono). Pregnant patients were followed using ultrasound scans, and P supplementation was maintained until the ninth gestational week.

Outcomes
Clinical pregnancy was determined by the visualization of a gestational sac with an embryo showing cardiac activity on ultrasound at weeks 5–7. Miscarriage was defined as a nonviable clinical pregnancy noted on ultrasound follow-up until gestational week 20. Live-birth rate was defined as the ratio between the number of deliveries resulting in at least one live birth and the number of ETs. The main outcome measures were the percentage of testicular and ejaculated spermatozoa containing fragmented DNA (%DFI), clinical pregnancy rates, miscarriage rates, and live-birth rates.

Statistical Analysis
Data are presented as mean ± SD or as percentages. A comparison of the quantitative variables was performed using the Mann–Whitney U test. For categorical data, the contingency Pearson χ² was used. P < .05 was considered statistically significant. A multivariate logistic regression model was constructed for dichotomous outcomes. For pregnancy outcomes, the covariates included male and female age, duration of infertility, and number of transferred embryos. Since no other comparative study with similar characteristics was available, we estimated a sample size of 80 subjects per group using these criteria for sperm identification and classification and a 5% significance level and 80% power. Statistical analyses were performed with the Statistical Package for Social Sciences (SPSS, ver.11).

RESULTS
Patient Characteristics
During the study period, 635 couples whose male partner had %DFI > 30% were admitted to the ART programs of participating institutions. Of them, 172 fulfilled the inclusion criteria and agreed to participate. Treatment options were discussed with the patients to whom we offered the use of testicular sperm. Eighty-one patients accepted this option and made up the TESTI-ICSI group. The remaining 91 opted to undergo ICSI with ejaculated sperm (EJA-ICSI group). Patient characteristics are shown in Table 1. Groups were homogeneous regarding age, endocrine profiles, infertility duration, and proportion of females with an associated fertility problem.

Testicular Sperm Retrieval
TESA was used in 52 cases and TESE in 29 cases. The sperm retrieval success rate with TESA or TESE was 100%. The overall complication rate after retrievals was 6.2% and did not differ between TESA and TESE methods. Pain was the most common complaint (n = 4 patients), while two patients had moderate scrotal swelling after TESE.

SDF
All semen specimens reassessed for SDF on the day of oocyte pick-up exhibited %DFI > 30%. The %DFI was compared between ejaculated and testicular sperm in the TESTI-ICSI group. As shown in Figure 1 (eSlide VM01016), spermatozoa were easily distinguished from somatic cells in testicular specimens. Spermatozoa exhibiting red fluorescence with a green flagellum and haloes of chromatin dispersion represented the cells with fragmented DNA (Fig. 1A, arrow cap). In contrast, spermatozoa exhibiting red fluorescence with a green flagellum and haloes of chromatin dispersion represented the cells with nonfragmented DNA (Fig. 1A, arrow).

Using these criteria for sperm identification and classification, the %DFI in the semen were 40.7% ± 9.9% and 40.9% ± 10.2% in the TESTI-ICSI and EJA-ICSI groups, respectively (Mann–Whitney U test; P = .23) and 8.3% ± 5.3% in testicular specimens of patients from the TESTI-ICSI group (Mann–Whitney U test; P < .001).

Sperm Injection Outcomes
There were no significant differences in the number and maturity of retrieved oocytes between the groups. The two-

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<td><strong>Clinical characteristics of the couples subjected to ICSI with testicular and ejaculated sperm.</strong></td>
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<tr>
<td><strong>Characteristic</strong></td>
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<tr>
<td>Male age, y</td>
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<td>Female age, y</td>
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<tr>
<td>Basal FSH levels, IU/L</td>
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<tr>
<td>Infertility duration, y</td>
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<tr>
<td>No. of days of stimulation</td>
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<tr>
<td>Total gonadotropin dose, IU</td>
</tr>
<tr>
<td>No. of oocytes</td>
</tr>
<tr>
<td>Retrieved</td>
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<td>Metaphase II</td>
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<td>Two pronuclei fertilization rate (%)</td>
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*Note: Data presented as mean ± SD.*

The SCD method for assessment of SDF in testicular sperm. A variant of the Halosperm test (Halotech DNA) that combines a dual fluorescent cocktail probe to discriminate somatic cells from spermatozoa was used. Spermatozoa and somatic cells exhibit differences in the wavelength emission associated with each fluorochrome (green for proteins and red for DNA). Spermatozoa exhibit only red fluorescence on the sperm head owing to protamine removal, while nonsperm cells fluoresce yellow as a result of the combined emission of both fluorochromes (A). Spermatozoa exhibiting red fluorescence with a green flagellum and no halo of chromatin dispersion represented those with fragmented DNA (arrow cap). In contrast, spermatozoa exhibiting red fluorescence with a green flagellum and haloes of chromatin dispersion represented those with nonfragmented DNA (arrow). A somatic cell with its typical high protein and DNA contents and a spermatozoon with its characteristic low protein remnant and high DNA content are seen in panels B and C, respectively, using a single channel fluorescence emission. After merging the information provided by protein and DNA selective staining, somatic cells and spermatozoa can be easily distinguished (D and D'). In addition, the sperm tail fluoresces in green, and this feature also helps to distinguish spermatozoa from other cell elements (A and A'). A high-resolution version of this slide for use with the Virtual Microscope is available as eSlide VM01016.
pronuclear zygote rate was lower in the TESTI-ICSI group (56.1%) compared with in the EJA-ICSI group (69.4%; \( P = .0001 \); Table 1). The quality embryos rate and the mean number of transferred embryos were similar between the groups (Table 2).

Pregnancy and miscarriage rates favored the TESTI-ICSI group (Table 2). The adjusted relative risk for miscarriage and live birth between the TESTI-ICSI and EJA-ICSI groups were 0.29 (95% confidence interval [CI], 0.10–0.82; Wald \( P = .019 \)) and 1.76 (95% CI, 1.15–2.70; Wald \( P = .008 \)). The number needed to treat by TESTI-ICSI compared with EJA-ICSI to obtain an additional live birth per fresh transfer cycles was 4.9 (95% CI, 2.8–16.8). A total of eight fresh transfers were cancelled, including four in the TESTI-ICSI group and four in the EJA-ICSI group. The reasons for cancellation were risk of ovarian hyperstimulation in six cases and no embryos available for transfers owing to arrested development in two cases. All embryos were vitrified in the first case.

### DISCUSSION

To our knowledge, this is the first prospective comparative study evaluating the use of testicular and ejaculated spermatozoa from oligozoospermic infertile men with high SDF on ICSI outcomes. The SDF index was approximately 5-fold lower in testicular sperm compared with in ejaculated sperm, and our results indicate that the use of testicular sperm was associated with improved ICSI outcomes in men with oligozoospermia and persistent elevated SDF. Given the markedly lower %DFI in testicular specimens compared with in ejaculated counterparts, the probability of selecting spermatozoa free of (or with reduced) DNA damage for ICSI will increase if testicular specimens are used. Fertilization of an oocyte by a genomically intact testicular spermatozoon will increase the chances of creating a normal embryonic genome that will ultimately increase the likelihood of pregnancy and live birth. The presence of nonfragmented paternal DNA may explain the observed better pregnancy outcomes with testicular sperm injections.

Our study depended primarily on testicular sperm retrieval, which yielded motile sperm for injections in all cases. We adopted the strict criterion of not including patients with severe oligozoospermia (<5 million/mL) because the presumably low number of retrieved spermatozoa might compromise the SDF assessment in testicular sperm. Both TESA and TESE were effective methods for sperm extraction in our group of men with oligozoospermia and high ejaculated SDF. A small portion of testicular tissue was obtained either by needle aspiration or by open biopsy. Because all patients had patent canicular systems, we used testicular rather than epididymal retrievals to avoid the risk of ductal obstruction. Moreover, it is our opinion that testicular retrievals should be preferred over epididymal retrievals based on the higher reported %DFI in epididymal sperm compared with in testicular sperm in men with obstructive azoospermia (27), even though these findings have not yet been confirmed in oligozoospermia. Although testicular retrievals are invasive procedures carrying the risk of complications such as hematoma and infection, no patient was affected in our series.

While it has been shown that the SDF measurement in consecutive ejaculates is associated with low biological variability (28), we included men with high SDF in at least two tests to avoid enrolling individuals with significant SDF variations. We also asked patients to abstain from ejaculation for a fixed period of 2–3 days before collection owing to the reported influence of the abstinence period on SDF test results (10). Moreover, all subjects had persistent high ejaculated SDF despite taking oral antioxidants. These agents have been shown to alleviate reactive oxygen species (ROS)-induced damage during sperm transport through the epididymis (29). ICSI rather than IVF was our chosen treatment method owing to the significantly higher pregnancy rates reported with ICSI in patients with high sperm DNA damage (30).

Prior to this study, Greco et al. were the first to describe the synergistic combination of testicular sperm retrieval and ICSI in infertile men with high SDF (17). In their study, a significantly higher pregnancy rate was obtained when sperm injections were carried out with testicular compared with ejaculated spermatozoa (44.4% vs. 6%; \( P < .05 \)). The data reported by Greco et al. suggest that infertile couples whose male partners have ejaculates with high SDF benefit from ICSI using testicular sperm. However, their findings should be interpreted with caution as they were based on a small group of 18 patients subjected to sequential ICSI attempts with ejaculated and testicular sperm. In another small study involving 17 patients with cryptozoospermia who failed to

### TABLE 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>TESTI-ICSI (( n = 77 ))</th>
<th>EJA-ICSI (( n = 87 ))</th>
<th>( P ) value</th>
<th>Relative risk (95% CI)</th>
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<tbody>
<tr>
<td>Embryos</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Number, mean ± SD</td>
<td>7.0 ± 3.7</td>
<td>6.4 ± 3.7</td>
<td>.327</td>
<td>NA</td>
</tr>
<tr>
<td>High quality on day 3 (%)</td>
<td>45.2 ± 12.0</td>
<td>41.8 ± 14.1</td>
<td>.118</td>
<td></td>
</tr>
<tr>
<td>No. transferred, mean ± SD</td>
<td>2.0 ± 0.3</td>
<td>1.9 ± 0.6</td>
<td>.206</td>
<td></td>
</tr>
<tr>
<td>Clinical pregnancy, n (%)</td>
<td>40 (51.9)</td>
<td>35 (40.2)</td>
<td>.131</td>
<td>1.29 (0.92–1.80)</td>
</tr>
<tr>
<td>Miscarriage, n (%)</td>
<td>4 (10.0)</td>
<td>12 (34.3)</td>
<td>.012</td>
<td>0.29 (0.10–0.82)</td>
</tr>
<tr>
<td>Live birth, n (%)</td>
<td>36 (46.7)</td>
<td>23 (26.4)</td>
<td>.007</td>
<td>1.76 (1.15–2.70)</td>
</tr>
</tbody>
</table>

Note: NA – not applicable.

*Among the enrolled patients (see Table 1), a total of eight fresh transfers were cancelled, including four in the TESTI-ICSI group and four in the EJA-ICSI group.*

conceive after repeated ICSI cycles using ejaculated sperm, Ben-Ami et al. showed that testicular sperm was associated with higher delivery rates compared with ejaculated sperm (27.5% vs. 9.4%; *P* = .028), but SDF was not assessed in their study [31]. Lastly, Weissman et al. showed that testicular spermatozoa was useful to overcome long-standing recurrent implantation failure in a group of four couples who failed to conceive by ICSI using ejaculated sperm [32].

A plausible reason for the improved ICSI outcomes in the aforementioned series might be the lower SDF levels in testicular specimens compared with ejaculated counterparts [17, 18]. In the study by Greco et al., DFI assessed by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) assay was significantly higher in ejaculated compared with testicular sperm (23.6% ± 5.1% vs. 4.8% ± 3.6%; *P* < .001) [17]. Using the same assay, Moskovicstev et al. studying a group of 12 men with high SDF found that the DFI was threefold higher in ejaculated sperm compared with testicular sperm (39.7% ± 14.8% vs. 13.3% ± 7.3%; *P* < .001) [18]. Interestingly, all testicular specimens but one in each study exhibited markedly lower %DFI than ejaculated counterparts. Our results are consistent with these observations as the %DFI measured by the SCD method was ≤ 15% in all testicular specimens with no exceptions. In contrast, %DFI was >30% in all ejaculated specimens. Whereas DNA damage can be found in testicular sperm as a result of direct meiotic failure or early defective chromatin assembling at the spermatid stage, the vast majority of DNA damage is observed in ejaculated sperm, thus indicating that SDF is mainly a post-testicular event [10, 27, 33, 34].

Importantly, we used the SCD method combining a dual fluorescent probe to target both the DNA and proteins. This method allows us to discriminate spermatozoa from other cell elements in testicular suspensions. A clear differentiation between sperm and nonsperm cells is possible because most protamine residues are removed after protein depletion in spermatozoa, while histones that are abundant in nonsperm cells are virtually unaffected [22]. The assessment of SDF in testicular specimens using this method is convenient when compared with the more complex assays, including TUNEL, sperm chromatin structure assay, and Comet. While it takes approximately 1.5 hours to assess SDF by SCD, the analysis would be 4 times longer using the technically challenging TUNEL assay [4]. Additionally, the SCD test is more sensitive than TUNEL for the evaluation of SDF [4], while flow-cytometry techniques and the Comet assay are not fully operative in tissues because spermatozoa are mixed with somatic cells [18]. Hence, protocol modifications will be required to first remove nonsperm cells, which may also eliminate spermatozoa, thus biasing the correct estimate of the true nature of SDF [35].

The applicability of the SCD test in testicular specimens was originally reported by Meseguer et al. to study the testicular sperm of men with azoospermia [36]. Using bright-field microscopy, the investigators showed that the method was operative in testicular specimens and noted that %DFI was significantly higher in men with nonobstructive azoospermia compared with men with obstructive azoospermia. Yet the DFI results obtained in their study with the SCD method were not compared to other assays. A few years ago, our group tested the validity of the SCD test in testicular sperm by studying a patient with Kartagener syndrome and recurrent fertilization failure [22]. We used the SCD test as described in the present study and compared test results to those obtained with the twodimensional Comet assay. The %DFI obtained with both methods was similar (SCD, 76.4%; Comet, 85.2%). These results were reassuring because the rationale of the SCD method is a two specific step process, namely, [1] controlled DNA denaturation and [2] controlled protamine depletion. Since the level of protamination in ejaculated and testicular sperm can be slightly different, it would therefore be possible that the SCD reagents generated different patterns in the chromatin structure. Additionally, in this case, we took advantage of the sample and performed a parallel DNA breakage detection-fluorescence in situ hybridization (DBD protocol) to assess DNA damage, which is a highly specific protocol based on in situ DNA hybridization using whole genomic DNA probes.

Test results with the DBD method have been previously shown to be equivalent to those of SCD [21]. In our aforementioned case study, testicular sperm labeled with DBD, which indicated the presence of DNA breaks, corresponded to those spermatozoa with small or no halo (indicative of DNA fragmentation by the SCD test). Despite not being validated in a large population, our group’s above-mentioned experimental approach corroborated Meseguer et al.’s observations indicating that SCD was operative in testicular specimens and added to them by showing that test results were comparable to those obtained by both the two-dimensional Comet and DBD assays. Nevertheless, further validation of the SCD test results in testicular sperm is warranted.

It is well known that testicular sperm are more vulnerable to DNA damage owing to the fact that sperm chromatin protamines are not fully cross-linked by disulphide bonds until sperm traverse the epididymis [37]. Previous studies have postulated that one of the mechanisms involved in SDF is oxidative stress–induced DNA damage during comigration of mature sperm with ROS-producing immature sperm through the epididymis [33, 34, 38–40]. Exposure of mature testicular sperm to ROS, produced either by immature sperm or epithelial cells lining the epididymis, can result in sperm damage before disulphide cross-linking takes place [41]. In fact, infertile men with oligozoospermia were found to have significantly higher oxidative parameters in the semen than fertile men [42]. The increased levels of ROS in these subjects have been associated with environmental and lifestyle factors, advanced age, obesity, infection, and other diseases [40, 43]. Although oral antioxidant intake has been commonplace, its effects to alleviate ROS-induced SDF are limited, and many patients still persist with high SDF after prolonged therapy [18]. Comparing antioxidants to placebo or no treatment, SDF rates were shown to be reduced by only 13.8% (95% CI, 10.4 to 17.7; *P* < .0001) [29]. Therefore, the use of testicular spermatozoa retrieved from men with complete spermatogenesis, which may be exempted from the oxidatively induced DNA damage taking place in the epididymis, becomes an attractive alternative.

In our study, fertilization rates after sperm injections with testicular sperm were lower than those with ejaculated sperm. Similar results have been observed when azoospermic...
Importantly, current evidence concerning the neonatal profile of babies born after ICSI using surgically retrieved gametes are reassuring (44, 45), but these data exclusively concern the subset of patients withazoospermia. The limited evidence favoring ICSI outcomes with the use of testicular sperm in men with high SDF calls for continuous monitoring until the safety of this strategy is confirmed. Any genetic and epigenetic effects in the offspring will require a more extensive investigation and long-term follow-up. Lastly, given the nonrandomized nature of our trial, it is possible that some bias exists, despite the homogeneity of the two cohorts compared. Therefore, further research is needed to confirm our findings.

In conclusion, our data indicate that the use of testicular sperm was associated with improved ICSI outcomes in men with oligozoospermia and persistent high SDF. Consideration should be given to testicular sperm retrieval when performing ICSI in such cases, provided less invasive treatments for alleviating DNA damage have failed.

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