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INVITED REVIEW

Assisted Reproductive Technology

# Use of testicular sperm for intracytoplasmic sperm injection in men with high sperm DNA fragmentation: a SWOT analysis

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Spermatozoa retrieved from the testis of men with high levels of sperm DNA fragmentation (SDF) in the neat semen tend to have better DNA quality. Given the negative impact of SDF on the outcomes of Assisted Reproductive Technology (ART), an increased interest has emerged about the use of testicular sperm for intracytoplasmic sperm injection (Testi-ICSI). In this article, we used a SWOT (strengths, weaknesses, opportunities, and threats) analysis to summarize the advantages and drawbacks of this intervention. The rationale of Testi-ICSI is bypass posttesticular DNA fragmentation caused by oxidative stress during sperm transit through the epididymis. Hence, oocyte fertilization by genomically intact testicular spermatozoa may be optimized, thus increasing the chances of creating a normal embryonic genome and the likelihood of achieving a live birth, as recently demonstrated in men with high SDF. However, there is still limited evidence as regards the clinical efficacy of Testi-ICSI, thus creating opportunities for further confirmatory clinical research as well as investigation of Testi-ICSI in clinical scenarios other than high SDF. Furthermore, Testi-ICSI can be compared to other laboratory preparation methods for deselecting sperm with damaged DNA. At present, the available literature supports the use of testicular sperm when performing ICSI in infertile couples whose male partners have posttesticular SDF. Due to inherent risks of sperm retrieval, Testi-ICSI should be offered when less invasive treatments for alleviating DNA damage have failed. A call for continuous monitoring is nonetheless required concerning the health of generated offspring and the potential complications of sperm retrieval.

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## INTRODUCTION

Male infertility is usually associated with the presence of abnormal semen parameters.<sup>1,2</sup> However, sperm dysfunctions other than those revealed by conventional semen analysis may be responsible for the difficulty to conceive.<sup>3,4</sup> Sperm DNA plays a critical role in normal embryo development as the genetic information passed on to the next generation depends on sperm DNA integrity.<sup>5,6</sup> Among DNA lesions, two main types are of utmost clinical importance, namely single-strand (SS-DB) and double-strand DNA breaks (DS-DB).<sup>6</sup>

Sperm DNA fragmentation (SDF), a broad term encompassing both SS-DB and DS-DB, is more common in infertile patients than in fertile counterparts.<sup>7</sup> Several etiological factors have been implicated in the impairment of sperm DNA content, including environmental and lifestyle factors, varicocele, male accessory gland infections, advanced paternal age, and systemic diseases.<sup>8–11</sup> Furthermore, recent evidence indicates that elevated SDF is associated with poor assisted reproductive outcomes.<sup>12</sup> Although sperm with fragmented DNA may fertilize an egg with apparently similar efficiency as sperm without DNA fragmentation, the negative impact of a damaged paternal chromatin to the integrity of embryonic genome is usually observed

after implantation.<sup>13</sup> This type of damage is often manifested by early pregnancy loss.<sup>11</sup> It has been speculated that SDF might also lead to a higher risk of congenital disabilities in the offspring.<sup>14,15</sup>

The most commonly used tests to measure DNA fragmentation in human sperm are terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), sperm chromatin dispersion test (SCD), single cell gel electrophoresis (Comet) assay, and sperm chromatin structure assay (SCSA). These methods measure the proportion of sperm with either real DNA breaks (e.g., TUNEL) or a combination of real DNA breaks and potentially denaturable DNA due to the preexistence of SS-DB or DS-DB (e.g., SCSA, SCD, and Comet). Probes or dyes are used to identify DNA breaks with the aid of fluorescence microscopy, optical microscopy, and flow cytometry according to the method type. These assays, however, are not interchangeable as they measure different aspects of SDF – though they are interrelated to a greater or lesser extent through properties of the DNA. Comprehensive reviews as regard the characteristics of SDF testing can be found elsewhere.<sup>10,16,17</sup>

SDF testing has been proposed as complementary to the information provided by routine semen analysis.<sup>6,12,18,19</sup> Moreover, the values of SDF can be clinically informative for Assisted Reproductive

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Technology (ART) outcomes, as discussed above and recently acknowledged by the Practice Committee of the American Society for Reproductive Medicine.<sup>2</sup> It is therefore suggested that SDF testing reflected the quality of the entire semen specimen, not just the damaged sperm detected in the test result.

In the presence of abnormal values, several strategies have been proposed to overcome SDF in couples undergoing ART. Varicocele repair,<sup>20</sup> oral antioxidant therapy,<sup>21</sup> short ejaculatory abstinence periods<sup>22</sup> and recurrent ejaculations,<sup>23</sup> and laboratory sperm selection techniques such as magnetic cell sorting (MACS),<sup>24</sup> physiological intracytoplasmic sperm injection (PICSI),<sup>25</sup> and intracytoplasmic morphologically selected sperm injection (IMSI)<sup>26</sup> have been attempted. Although some interventions might be potentially useful, including removal of highly apoptotic sperm by MACS and alleviation of oxidative status by oral antioxidants in men with low antioxidant levels, none of them, alone or combined, have been unequivocally proven to fully bypass the potential detrimental effect of abnormal SDF on ART outcomes.<sup>2</sup> Notwithstanding, the methods employed to reduce SDF are usually applied to an unselected population of men undergoing ART regardless of SDF rates. A strict inclusion criterion, for instance, of only men with high SDF and grouped by etiology might have avoided diluting the effect size of some of these techniques, a hypothesis that deserves further investigation.

Recently, the use of testicular rather than ejaculated sperm for intracytoplasmic sperm injection (ICSI) among men with high SDF has gained increased attention due to reports of better pregnancy outcomes.<sup>27,28</sup> In this review, we summarize the studies that have been published since the first report of the use of testicular sperm for ICSI (Testi-ICSI) among men with high SDF. Moreover, we aim to explore the biological plausibility as regards the potential advantages of Testi-ICSI and try to offer suggestions for its safe use and directions for future research. For this, we applied the SWOT (strengths, weaknesses, opportunities, and threats) analysis, a method usually used in business but also adaptable to reproductive medicine.<sup>29</sup> The SWOT analysis framework helps focus on the strengths, understand the weaknesses that can assist in managing and minimizing the threats, and take the greatest possible advantage of the opportunities available (Figure 1).

## STRENGTHS

### *Testicular sperm has better chromatin integrity than ejaculated sperm*

Moskovtsev *et al.*<sup>30</sup> evaluated DNA damage in ejaculated and testicular spermatozoa of 12 men with persistently high DNA damage despite taking oral antioxidants for 3 months. They compared the values of DNA fragmentation assessed by TUNEL between testicular sperm obtained by testicular sperm extraction (TESE) and ejaculated

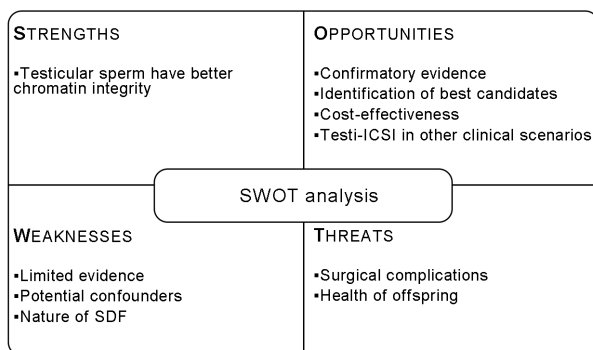
sperm collected from the same patients on the same day. The rates of SDF in ejaculated sperm were 3-fold higher than testicular sperm ( $39.7\% \pm 14.8\%$  vs  $13.3\% \pm 7.3\%$ ,  $P < 0.001$ ). Using the Comet assay, Steele *et al.*<sup>31</sup> showed that the percentage of sperm with intact chromatin was higher ( $83.0\% \pm 1.2\%$ ) in testicular specimens of twenty men with obstructive azoospermia than in proximal epididymal counterparts ( $75.4\% \pm 2.3\%$ ;  $P < 0.05$ ). Along the same lines but using an experimental mice model, Sukanuma *et al.*<sup>32</sup> observed that the passage of sperm through the epididymis was associated with a loss of sperm DNA integrity and fertilizing capacity. The characteristics of the studies mentioned above are summarized in Table 1.

These findings indicated that the main pathways leading to SDF are triggered either during sperm transport through the seminiferous tubules or during the epididymis transit, or both.<sup>33</sup> The plausibility of this biological phenomenon relies on three important facts. First, chromatin compaction is still ongoing during epididymal transit. Second, excessive ROS can be generated in the epithelial cells of epididymis under physicochemical stressors such as high temperature and environmental conditions.<sup>34–36</sup> Finally, some endonucleases can cleave DNA of mature live sperm.<sup>37</sup> As a result, sperm DNA damage may ensue through different pathways, including hydroxyl radical, nitric oxide, and activation of sperm caspases and endonucleases, thus explaining the high positivity for SDF in live ejaculated sperm.<sup>33</sup> This oxidative-induced damage to the sperm chromatin can be potentially avoided in ICSI candidates provided the epididymis is bypassed. Hence, the use of testicular sperm harvested by testicular sperm aspiration (TESA) or extraction (TESE) becomes attractive as the probability of selecting spermatozoa free of DNA damage for ICSI can be increased. Likewise, the fertilization of an oocyte by a genomically intact testicular spermatozoon will improve the chances of creating a normal embryonic genome that will ultimately increase the likelihood of pregnancy and live birth.<sup>27</sup>

### *Testi-ICSI has been associated with improved assisted reproductive technology outcomes*

The first report concerning the use of testicular rather than ejaculated sperm for ICSI was published in 2005.<sup>38</sup> The authors evaluated 18 couples who had at least two previous unsuccessful ICSI cycles with ejaculated sperm and whose semen evaluation showed  $>15\%$  spermatozoa with fragmented DNA assessed by TUNEL. Testicular sperm was obtained by testis biopsy, and SDF was evaluated on prepared smears in a similar manner as ejaculated sperm smears. However, in the second ICSI attempt, all sperm injections were performed with testicular sperm. The mean  $\pm$  s.d. SDF rates in testicular sperm and ejaculated sperm were  $4.8\% \pm 3.6\%$  and  $23.6\% \pm 5.1\%$ , respectively ( $P < 0.001$ ). There were no significant differences in fertilization and cleavage rates or in the proportion of embryos with good morphology when the first and second ICSI attempts were compared. However, whereas only one pregnancy that spontaneously aborted was obtained in the cycles with ejaculated sperm, eight clinical pregnancies (four singletons and four twins) have been achieved in the cycles carried out with testicular sperm. No miscarriages were recorded after Testi-ICSI.

In 2010, Sakkas and Alvarez showed that pregnancy outcomes were improved using testicular sperm rather than ejaculated sperm in patients with high levels of SDF.<sup>39</sup> These authors studied 72 patients with  $>20\%$  SDF by TUNEL and found higher implantation ( $P = 0.0021$ ) and clinical pregnancy rates ( $P = 0.035$ ) and lower miscarriage rates in ICSI cycles performed with testicular sperm. Recently, Mehta *et al.*<sup>28</sup> reported a case series of 24 men with oligozoospermia ( $<5 \times 10^6 \text{ ml}^{-1}$ ) and SDF  $>7\%$  by TUNEL with previous failed ICSI attempts with the use of ejaculated sperm. The patients were subjected to microdissection



**Figure 1:** A strength-weakness-opportunities-threats analysis of using testicular sperm for ICSI in infertile men with high sperm DNA fragmentation.

**Table 1: Characteristics of studies examining the relationship between sperm DNA fragmentation rates in ejaculated, epididymal, and testicular sperm**

Study	Design	Participants	SDF test used	Outcome measures	Main results
Steele <i>et al.</i> <sup>31</sup>	Cross-sectional study	Twenty men with OA and ten fertile men undergoing vasectomy (controls)	Comet	Values of SDF between paired testicular sperm and proximal epididymal sperm from men with OA, and SDF rates in ejaculated and testicular specimens of controls	Percentage of sperm with higher intact chromatin (83.0%±1.2%*) in testicular than proximal epididymal sperm (75.4%±2.3%); <i>P</i> <0.05) in OA No difference in percentage of sperm with intact chromatin between testicular specimens of OA men and both ejaculated (78.9%±3.9%) and testicular (86.8%±1.8%) specimens of controls
Suganuma <i>et al.</i> <sup>32</sup>	Experimental animal study	Wild-type and mutant infertile mice with deficiency in protamine 2 processing ( <i>Tnp1-/-Tnp2+/-</i> ) <sup>§</sup>	Acridine orange	Values of sperm DNA integrity in testicular, caput, and cauda epididymis of mutant and wild-type mice, and paired comparison of ICSI outcomes with the use of the three sperm sources	Mutant mice had lower percentages of sperm with intact chromatin both in the caput and cauda epididymis than normal mice When the motile sperm from the cauda epididymis of mutant mice were evaluated by ICSI, there were increases in chromosome abnormalities at the first cleavage division, and reductions in fertilization, development to the first cleavage division, and implantation of developing embryos compared to cauda sperm from wild-type mice and caput epididymal sperm from these mutant mice. There were no differences in the frequencies of implantation and development to term of embryos derived from testicular or caput epididymal sperm from <i>Tnp1-/-Tnp2+/-</i> males and from wild-type mice, thus indicating deterioration of the sperm genomic integrity during epididymal transit due to a reduced DNA protection in mutant mice
Moskovtsev <i>et al.</i> <sup>30</sup>	Prospective, observational, cohort study	12 men with persistently high DNA damage despite taking oral antioxidants for 3 months	TUNEL	Values of SDF between paired testicular sperm and ejaculated sperm	SDF rates lower in testicular than ejaculated sperm (39.7%±14.8% vs 13.3%±7.3%, <i>P</i> <0.001)

\*Standard error of the mean; §Sperm from *Tnp1-/-Tnp2+/-* mice show deficiency in protamine two processing despite approximately normal levels of protamines and a normal ratio of protamines one and two. Sperm from such mice exhibit lower disulfide cross-linking and reduced DNA protection. ICSI: intracytoplasmic sperm injection; TUNEL: terminal deoxyribonucleotide transferase-mediated dUTP nick-end labeling assay; SDF: sperm DNA fragmentation; OA: obstructive azoospermia

testicular sperm extraction (micro-TESE) and the retrieved sperm were used for ICSI. Clinical pregnancy was achieved in 50% of 24 couples in the Testi-ICSI cycle and all pregnancies resulted in deliveries of healthy babies. The mean TUNEL-positive level was 24.5% for ejaculated sperm and 4.6% for testicular sperm. In another recent study, Pabuccu *et al.*<sup>40</sup> retrospectively analyzed the outcomes of ICSI from 71 couples with repeated ART failures (>2 unsuccessful ICSI attempts), in which the male partner had normozoospermia (2010 WHO criteria) and high SDF (>30%) by TUNEL. Testicular and ejaculated sperm were used for ICSI in 31 and 40 couples, respectively. Despite similar fertilization and implantation rates, the clinical (41.9% vs 20.0%; *P* = 0.04) and ongoing (38.7% vs 15.0%; *P* = 0.02) pregnancy rates per started cycle were higher when testicular sperm were used in preference over ejaculated sperm.

In the only prospective comparative study published to date, Esteves *et al.*<sup>27</sup> compared treatment outcomes between ejaculated and testicular sperm by evaluating a cohort of 172 infertile men with elevated SDF subjected to ICSI. In this study, the authors enrolled infertile men with mild-to-moderate idiopathic oligozoospermia ( $5 \times 10^6$ – $15 \times 10^6$  spermatozoa per ml) presenting with persistent high SDF (>30%) despite oral antioxidant therapy with a combination of Vitamins C, E, folic acid, selenium, and zinc for 3 months. On the day of oocyte retrieval, SDF was assessed in all patients after 2–3 days of ejaculatory abstinence using the sperm chromatin dispersion (SCD) test. In the group of patients undergoing sperm retrieval, which was carried out by either TESE or TESA, SDF was also assessed in testicular specimens using the SCD method combining a dual fluorescent probe to target both the DNA and proteins. This approach allowed for discrimination between spermatozoa and other cell elements in

testicular suspensions. The rates of SDF in the group of men subjected to sperm retrieval were 5-fold higher in the semen ( $40.7\% \pm 9.9\%$ ) than in the testis ( $8.3\% \pm 5.3\%$ ; *P* < 0.001); in this group, all sperm injections were performed with testicular sperm. On the contrary, SDF rates were  $40.9\% \pm 10.2\%$  in the group of patients subjected to ICSI with ejaculated sperm. The comparison groups were similar concerning male and female demographic characteristics. However, the miscarriage rates were lower whereas the live birth rates were higher in the couples subjected to sperm injections with testicular sperm. The adjusted relative risk for miscarriage and live birth between testicular and ejaculated groups was 0.29 (95% CI: 0.10–0.82; *P* = 0.019) and 1.76 (95% CI: 1.15–2.70; *P* = 0.008), respectively. To our knowledge, this is the study published to date comparing SDF and ICSI outcomes in couples whose male partner had elevated SDF.

The characteristics of the studies discussed above are summarized in **Table 2**.

## WEAKNESSES

### Limited evidence

The available evidence favoring the use of testicular sperm for ICSI in cases with high SDF is still limited. Most studies have evaluated a small cohort of men or lacked a control group.<sup>28,38,39</sup> Moreover, the validity of comparing the results of repeat ICSI cycles with previous unsuccessful ones is scientifically questionable. Only one prospective comparative study, albeit not randomized, was powered to detect differences in live birth rates.<sup>27</sup> Notwithstanding, the available evidence may offer the opportunity to develop a potentially interesting systematic review and meta-analysis to compare ICSI outcomes for ejaculated versus testicular sperm among men with high sperm DNA fragmentation in semen.

**Table 2: Characteristics of studies examining the outcomes of ICSI after the use of testicular and ejaculated sperm among men with high SDF**

Study	Design	Participants	SDF test used	Outcome measures	Main results
Greco <i>et al.</i> <sup>38</sup>	Case-control study	18 couples with at least two previous unsuccessful ICSI attempts using ejaculated sperm Male partners with >15% spermatozoa with fragmented DNA (neat semen) All couples subjected to consecutive ICSI cycles with both ejaculated and testicular sperm	TUNEL	Values of SDF between paired testicular sperm and ejaculated sperm Fertilization, cleavage, and clinical pregnancy rates	Mean±s.d. percentage SDF lower in testicular sperm than ejaculated sperm (4.8%±3.6% vs 23.6%±5.1%, respectively; <i>P</i> <0.001) No differences in fertilization and cleavage rates or in the proportion of embryos with good morphology between the first and second ICSI attempts Higher pregnancy rates after sperm injections carried out with testicular than ejaculated spermatozoa (44.4% vs 6%; <i>P</i> <0.05)
Sakkas and Alvarez <sup>39</sup>	NR	72 couples with repeated IVF failure using ejaculated sperm Male partners with high SDF in neat semen (>20%); Eja-ICSI ( <i>n</i> =42); Testi-ICSI ( <i>n</i> =30)	TUNEL	Clinical pregnancy, implantation, and miscarriage rates after ICSI using ejaculated or testicular sperm	Higher CPR (40.0% vs 13.8%; <i>P</i> =0.03) and IR (28.1% vs 6.6%; <i>P</i> <0.002), and lower miscarriage rates (6.2% vs 75.0%; <i>P</i> =0.01) in ICSI cycles performed with testicular sperm
Mehta <i>et al.</i> <sup>28</sup>	Case series	24 couples with previous failed ICSI attempts using ejaculated sperm Male partners with oligozoospermia (<5×10 <sup>6</sup> ml <sup>-1</sup> ) and high SDF (>7%) in neat semen	TUNEL	Values of SDF between paired testicular sperm and ejaculated sperm Clinical pregnancy rates after Testi-ICSI	Mean TUNEL-positive score lower for testicular sperm (5%; 95% CI: 3%–7%) than ejaculated sperm (24%; 95% CI: 14%–34%; <i>P</i> =0.01) CPR: 50%; all pregnancies resulted in live births
Esteves <i>et al.</i> <sup>27</sup>	Prospective cohort	172 infertile couples on their first ICSI attempt Female infertility excluded and all male partners with oligozoospermia (5×10 <sup>6</sup> –15×10 <sup>6</sup> ml <sup>-1</sup> ) and persistent high SDF (>30%) after oral antioxidant therapy Testi-ICSI ( <i>n</i> =81); Eja-ICSI ( <i>n</i> =91)	SCD	Values of SDF between paired testicular sperm and ejaculated sperm in Testi-ICSI group Fertilization, cleavage, pregnancy rates, and miscarriage rates	Mean±s.d. SDF rates lower in testicular sperm than ejaculated sperm (8.3%±5.3%; vs 40.7%±9.9%; respectively; <i>P</i> <0.001) Lower 2PN fertilization rates in Testi-ICSI group (56.1%) than Eja-ICSI group (69.4%; <i>P</i> =0.0001); similar rates of high-quality embryos available for transfer on day 3 (45.2%±12.0% vs 41.8%±14.1%, respectively) CPR not statistically different between Testi-ICSI (51.9%) and Eja-ICSI (40.2%) groups, but lower miscarriage rates (10.0% vs 34.3%; <i>P</i> =0.01) and higher LBR (46.7% vs 26.4%; <i>P</i> =0.007) in Testi-ICSI than Eja-ICSI
Pabuccu <i>et al.</i> <sup>40</sup>	Retrospective cohort	71 couples with repeated ART failures (>2 unsuccessful ICSI attempts with ejaculated sperm), in which the male partner had normozoospermia (2010 WHO criteria) and high SDF (>30%)	TUNEL	Fertilization, implantation, clinical and ongoing pregnancy rates per started cycle	Similar 2PN fertilization rates (74.1%±20.7% vs 71.1%±26.9%) and IR (24.7% vs 15.0%) in Testi-ICSI and Eja-ICSI groups, respectively Higher CPR (41.9% vs 20.0%; <i>P</i> =0.04) and OPR (38.7% vs 15.0%; <i>P</i> =0.02) per started cycle in Testi-ICSI group than Eja-ICSI group
Bradley <i>et al.</i> <sup>41</sup>	Retrospective cohort	448 ICSI cycles in couples whose male partner had high SDF (≥29%) ICSI with ejaculated sperm and no intervention to deselect sperm with SDF ( <i>n</i> =80); Testi-ICSI ( <i>n</i> =146)	SCIT	Fertilization, clinical pregnancy, implantation, miscarriage, and live birth rates with fresh blastocyst transfers after ICSI using ejaculated or testicular sperm	Lower 2PN fertilization rates (57.0% vs 66.0%; <i>P</i> <0.001) with Testi-ICSI Higher IR (41.1% vs 24.0%; <i>P</i> <0.05), CPR (49.5% vs 27.5%; <i>P</i> <0.05), and LBR (43.7% vs 24.9%; <i>P</i> <0.05) in Testi-ICSI group than Eja-ICSI group No difference in miscarriage rates between Testi-ICSI and Eja-ICSI groups (13.2% vs 10.2%, respectively)

NR: not reported; CPR: clinical pregnancy rates; IR: implantation rates; LBR: live birth rates; SDF: sperm DNA fragmentation; ICSI: intracytoplasmic sperm injection; Eja-ICSI: ejaculated sperm used for sperm injections; Testi-ICSI: testicular sperm used for sperm injections; TUNEL: terminal deoxyribonucleotide transferase-mediated dUTP nick-end labeling assay; SCD: sperm chromatin dispersion test; SCIT: sperm chromatin integrity test, which is a variation of SCSA; ART: assisted reproductive technology; WHO: World Health Organization; 2PN: two-pronuclear zygote; SCSA: sperm chromatin sperm assay; CI: confidence interval; s.d.: standard deviation

### Potential confounders

The predictive value of SDF for ART success is confounded by several factors. Foremost among these is perhaps the issue of the oocyte quality, which is related to maternal age, and its capability of repairing DNA damage before the first cleavage.<sup>42</sup> Furthermore, sperm DNA quality deteriorates with advanced paternal age.<sup>43</sup> These factors may further exacerbate the deleterious effect of SDF in ART cycles performed in women of advanced age and in those with reduced ovarian reserve.<sup>44</sup> Importantly, these observations indicate that female factor infertility represents an relevant confounder to be controlled by strict inclusion criteria, subgroup analysis, or statistical methods.

### Nature of SDF

Testicular sperm may not always overcome the problem of SDF. It is well known that sperm DNA damage can occur in the seminiferous

tubules as a result of apoptosis or due to defects in chromatin remodeling during spermiogenesis.<sup>6</sup> Intratesticular apoptosis induced by an impairment in sperm maturation leads to early DNA damage; these spermatozoa go through the genital tract without being further damaged by oxidative stress.<sup>33</sup> Consequently, the advantage of testicular sperm over ejaculated sperm as regards decreasing SDF is likely to be restricted to posttesticular SDF.

## OPPORTUNITIES

### Confirmatory evidence

SDF has been shown to contribute to infertility in up to 30% of men.<sup>7,45</sup> Among couples undergoing ICSI, abnormally high levels of SDF are found in approximately 32% of the cases.<sup>14</sup> And despite being usually associated with abnormal semen parameters, a significant proportion of men (20%–40%) with otherwise normal semen parameters have high

SDF.<sup>46,47</sup> Therefore, a large proportion of infertile men to be enrolled in ART can potentially benefit from Testi-ICSI.

Still, the evidence concerning the advantage of using testicular over ejaculated sperm to overcome infertility in ICSI candidates with high seminal SDF values is limited, as already discussed. Hence, a call for confirmatory evidence using a prospective approach is needed. For instance, if the aim is to confirm a difference of 10% in the primary outcome measure (e.g., live birth rates), assuming this difference as clinically significant, a minimum of 770 patients (385 per group) will be required to have an 80% chance of detecting, as significant at the 5% level, an increase in live birth rates (i.e., from 30% live birth rates in the control group to 40% in the experimental group). The use of SDF testing and Testi-ICSI will represent an important clinical contribution to doctors and patients alike if the increased likelihood of success is confirmed in such trial, thus reassuring the validity of Testi-ICSI.

### Identification of best candidates for Testi-ICSI

Posttesticular damage during sperm transport through the epididymis plays a major role in SDF.<sup>39</sup> Infertile men with varicocele, for instance, often have higher SDF than their counterparts without varicocele. In such men, reactive oxygen and nitrogen species are released not only in endothelial cells of the dilated pampiniform plexus and testicular cells (developing germ cells, Leydig cells, macrophages, and peritubular cells), but also in the principal cells of the epididymis.<sup>48,49</sup> Apart from varicocele, the epididymis can be the origin of SDF in (1) infectious and inflammatory states that may contribute to chronic epididymal dysfunctions and (2) spermatogenesis defects associated with residual cytoplasm and defective protamination, or both. The former can be observed in spinal cord injury,<sup>50</sup> postvasectomy reversal,<sup>51</sup> and clinical or subclinical epididymitis.<sup>52</sup> In these cases, SDF may result from excessive ROS production by spermatozoa themselves in response to a more prolonged epididymal transit or infiltrating polymorphonuclear leukocytes, or both. The latter can be genetically determined or idiopathic and SDF results from the higher susceptibility of DNA to posttesticular degradation by endonucleases.<sup>53</sup> Besides, oxidative-induced SDF can also occur postejaculation. In fact, a strong association exists between the presence of male accessory gland infections (MAGI) and seminal ROS levels, and between smoking and excessive seminal plasma leukocytes and ROS; both conditions have been associated with high SDF.<sup>54,55</sup> Due to the lack of knowledge about the usefulness of Testi-ICSI in the particular clinical scenarios mentioned above, opportunities exist for investigating the outcomes of Testi-ICSI in subgroups of men more prone to posttesticular sperm DNA damage.

### Cost-effectiveness

Given the widespread availability of both SDF testing and urologists performing sperm retrieval, Testi-ICSI could be implemented in the majority of ART units unlike expensive technologies such as IMSI and MACS. In this regard, the cost-effectiveness of Testi-ICSI could be compared to other laboratory preparation methods to deselect sperm with damaged DNA. To our knowledge, there is only one study that compared interventions to deselect sperm with DNA damage. In this report, Bradley *et al.*<sup>41</sup> retrospectively evaluated 448 ICSI cycles from couples whose male partners had high levels of SDF. Sperm injections were performed with either ejaculated sperm or testicular sperm. In the ejaculated sperm group, the authors applied interventions to deselect sperm with DNA fragmentation, namely, IMSI and PICSI, and compared outcomes with a control group of “no intervention.” They also compared the outcomes of ICSI using ejaculated sperm with and without intervention to Testi-ICSI and found higher live

birth rates ( $P < 0.05$ ) with Testi-ICSI (49.8%) than IMSI (28.7%) and PICSI (38.3%). The lowest live birth rates (24.2%) were achieved when no intervention was carried out to deselect sperm with DNA fragmentation ( $P = 0.020$ ) (Table 2). Unfortunately, this study neither provides data on the reduction of SDF rates by each intervention modality nor evaluates the cost per delivery by each intervention investigated.

Yet, the study of Esteves *et al.*<sup>27</sup> has shown that the number needed to treat (NNT) by testicular compared to ejaculated sperm to obtain an additional live birth per fresh transfer cycles was 4.9 (95% CI: 2.8–16.8). In other words, if we need to treat about five patients with Testi-ICSI to obtain an additional pregnancy per transfer, it means we could avoid one out of five oocyte pick-ups. Obviously, this simplistic estimation does not consider the additional contribution of frozen embryos regarding cumulative pregnancy rates. Apart from the medical benefit, it is equally important to evaluate the economical advantage of a given intervention. Along the same lines, a predictive model could be developed taking into consideration the differences in specific costs per procedures that may differ between clinics and countries. As such, Testi-ICSI seems to be an attractive method to overcome infertility associated with high SDF in need of an in-depth cost-effectiveness analysis.

### Cryptozoospermia

Apart from SDF, the use of testicular sperm for ICSI in other scenarios has been poorly studied. To date, only four reports accounting for <300 cycles assessed ICSI outcomes between ejaculated and testicular sperm in cryptozoospermic men.<sup>56–59</sup> Ben-Ami *et al.*<sup>56</sup> reported higher implantation rate (20.7% vs 5.7%;  $P = 0.003$ ), higher pregnancy rate (42.5% vs 15.1%;  $P = 0.004$ ), and higher delivery rate (27.5% vs 9.4%;  $P = 0.028$ ) with testicular sperm in a small cohort of 17 cryptozoospermic men who had undergone multiple unsuccessful ICSI cycles with ejaculated sperm. In another study, Hauser *et al.*<sup>57</sup> studied 13 couples whose male partner had virtual azoospermia or cryptozoospermia subjected to multiple ICSI cycles with ejaculated and fresh and frozen testicular sperm. In this study, fertilization rates (50.0% vs 38.2%,  $P < 0.05$ ), high-quality embryo rate (65.3% vs 53.2%,  $P < 0.05$ ), and implantation rates (18.1% vs 5.1%;  $P = 0.04$ ) favored fresh testicular sperm compared with ejaculated sperm. Along the same lines, Bendikson *et al.*<sup>58</sup> reported a trend toward higher clinical pregnancy rates for testicular sperm in 16 couples undergoing ICSI with ejaculated and testicular sperms. On the contrary, Amirjannati *et al.*<sup>59</sup> showed no differences in fertilization rates and embryo quality between couples undergoing ICSI with ejaculated (208 cycles) and testicular sperms (16 cycles), but pregnancy rates were not taken into account. Recently, the data from these aforementioned studies were summarized in a meta-analysis, which concluded that there were no differences in ICSI pregnancy rates (relative risk [RR]: 0.53, 95% CI: 0.19–1.42,  $I^2 = 67%$ ) or fertilization rates (RR: 0.91, 95% CI: 0.78–1.06,  $I^2 = 73%$ ) between testicular and ejaculated sperm groups.<sup>60</sup> The authors concluded that use of testicular sperm rather than ejaculated sperm for ICSI in men with cryptozoospermia is not recommended. However, the included studies have many limitations. Apart from being underpowered to detect clinically significant differences, only one of them has considered live birth rates as the primary outcome. Therefore, the verdict of this meta-analysis should be taken with caution until further sufficiently sized and properly designed studies are developed.

### Repeated implantation failure and recurrent pregnancy loss

The published data concerning Testi-ICSI in repeated implantation failure are merely anecdotal. A single report described success with

testicular sperm in four couples with multiple ICSI failures.<sup>61</sup> As far as recurrent pregnancy loss (RPL) is concerned, the plausibility of Testi-ICSI relies on the positive association between high SDF and miscarriage in IVF/ICSI cycles. In a meta-analysis evaluating 2969 couples, the risk of miscarriage was increased by 2.2 fold when semen specimens with an abnormally high proportion of DNA damage were used for ICSI (95% CI: 1.54–3.03;  $P < 0.00001$ ).<sup>15</sup> In another meta-analysis pooling data from 14 studies, elevated SDF was associated with higher miscarriage rates in ICSI cycles (OR: 2.68; 95% CI: 1.40–5.14;  $P = 0.003$ ).<sup>62</sup> However, none of the studies included in the meta-analyses mentioned above have specifically investigated patients with RPL.

On the contrary, a recent report examined SDF rates by TUNEL among male partners of 112 couples experiencing RPL and control groups of infertile men with abnormal semen parameters and fertile men with normal semen parameters according to the WHO criteria.<sup>63</sup> Despite normal semen analysis, SDF was higher in the RPL group compared with fertile controls ( $18.8\% \pm 7.0\%$  vs  $12.8\% \pm 5.3\%$ ,  $P < 0.001$ ) and similar to those of infertile patients ( $20.8\% \pm 8.9\%$ ). The authors also reported a significant positive correlation between the number of RPL events and an elevated level of SDF. Despite this, to our knowledge, there are no published studies specifically investigating the role of testicular sperm in ART patients with RPL.

## THREATS

### *Surgical complications*

Sperm retrievals require surgical interventions that are usually carried out on an outpatient basis. The recovery period is 24–72 h, and risks are low but include infection (<1%), hematoma (<5%), and testicular atrophy.<sup>64</sup> The potential for intratesticular bleeding after testicular sperm retrieval seems to be higher with open than percutaneous biopsy techniques.<sup>65</sup> The more problematic adverse effect of sperm retrieval, namely, reduction in testosterone production, is caused by the removal of large amounts of testis tissue containing hormone-producing Leydig cells with open surgical techniques (TESE). This effect has been reported in a few men with nonobstructive azoospermia subjected to multiple biopsies.<sup>66</sup> However, from a holistic viewpoint, less invasive treatments for men (i.e., ICSI with ejaculated sperm) might represent more invasive treatments for women (i.e., repeat oocyte retrievals) if fewer pregnancies and/or more miscarriages are obtained with ejaculated sperm among men with high SDF.

### *Health of offspring*

While defective spermatozoa passing the testicular barrier can be deselected through natural apoptotic-like process,<sup>33</sup> it is possible that defective testicular sperm originating from a subpopulation that would be blocked in its ontogeny during the maturation process is selected for ICSI. Aneuploidy rates were higher in testicular sperm obtained from men with nonobstructive azoospermia than epididymal sperm and ejaculated sperm.<sup>67,68</sup> Whereas testicular spermatozoa have overall low DNA damage, this potential advantage could be offset by the higher aneuploidy rates. In one study, Moskovtsev *et al.*<sup>69</sup> compared aneuploidy rates at the testicular and posttesticular levels in the same patients with high SDF. Although SDF rates were almost 3-fold lower in testicular sperm ( $40.6\% \pm 14.8\%$  vs  $14.9\% \pm 5.0\%$ ,  $P < 0.05$ ), higher aneuploidy rates for chromosomes 18, 21, X, and Y were observed in testicular spermatozoa than ejaculated sperm ( $12.41\% \pm 3.7\%$  vs  $5.77\% \pm 1.2\%$ ,  $P < 0.05$ ).<sup>69</sup> Still, the proportion of testicular sperm with aneuploidy was relatively low, and these findings are yet to be confirmed in larger series comprising both men with oligozoospermia and unexplained infertility. Notwithstanding, it might be argued that ICSI candidates

represent a particular category of patients that would be unlikely to attain natural reproduction. Therefore, a small increase in the risk of having health issues in the offspring could be acceptable in return of a confirmed beneficial effect of Testi-ICSI, provided the actual number of affected individuals is extremely low. Since the evidence favoring ICSI outcomes with the use of testicular sperm in men with high SDF is limited, a call for continuous monitoring is warranted until the safety of this strategy is confirmed further. In fact, any genetic and epigenetic effects in the offspring will require a more extensive investigation and long-term follow-up.

## CONCLUSIONS

Fair evidence indicates that sperm DNA fragmentation is associated with poorer ART outcomes. There is a rationale for the use of testicular sperm for ICSI owing to the improvement in live birth rates in men with persistent high SDF in semen. The biological plausibility of this favorable effect relates to the fact that posttesticular exposure of spermatozoa to oxidative DNA damage in the epididymis is avoided. However, the evidence favoring Testi-ICSI in such cases is still limited as there are no randomized controlled trials comparing ejaculated and testicular sperm. And notably, the current literature does not support the use of testicular in preference over ejaculated sperm for ICSI in clinical scenarios other than high SDF, including extremely low sperm numbers. Despite the potential risks associated with sperm retrieval, ample opportunities exist to confirm the efficacy of Testi-ICSI and further investigate the role of testicular sperm for ICSI in different subgroups of infertile men with and without high DNA damage. Furthermore, the cost-effectiveness of Testi-ICSI as regards the reduction in SDF can be compared with other laboratory methods of sperm selection. At present, the method should be reserved for selected men who have failed less invasive treatments for known and unknown causes of sperm DNA damage, particularly when posttesticular sperm DNA damage is suspected.

## REVIEW CRITERIA

We searched PubMed until December 2016 to identify studies evaluating ICSI outcomes among men with high levels of SDF, irrespective of test method and cutoff values utilized, who underwent either consecutive ICSI cycles using both ejaculated sperm and surgically extracted testicular sperm or separate ICSI cycles using ejaculated sperm or testicular sperm. Our search was based on the following key words, alone or combined: “intracytoplasmic sperm injection,” “sperm DNA fragmentation,” “sperm DNA damage,” “sperm chromatin integrity OR damage,” “oligozoospermia,” “normozoospermia,” “testicular OR testicular sperm,” “ejaculated OR ejaculated sperm,” with the filters: “humans,” “English language,” and “Full text.” We excluded studies involving men with a diagnosis of azoospermia and those in which SDF analysis had not been performed. Our search identified five studies, which are summarized in **Table 1**.

## AUTHOR CONTRIBUTIONS

SCE and MR designed the study, participated in the acquisition of data, and drafted the manuscript. NG revised the manuscript and helped in data interpretation and coordination. All authors read and approved the final manuscript.

## COMPETING INTERESTS

The authors declare no competing interests.

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