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The Use of Testicular Sperm for Intracytoplasmic Sperm Injection in Patients with High Sperm DNA Damage: A Systematic Review

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The advent of intracytoplasmic sperm injection (ICSI) has changed the human reproduction landscape by overcoming several limitations related to both male and female infertility factors. However, despite the development of new technologies, the live-birth rate with ICSI has not exceeded 30%. In order to improve assisted reproductive technology outcomes, advanced sperm function analysis have gained increased attention and the effects of sperm DNA fragmentation (SDF) on assisted reproduction success are being extensively studied. Utilizing ejaculated sperm with an elevated SDF has been found to result in poor ICSI outcomes. Furthermore, studies have reported that testicular sperm has lower SDF level, when compared to ejaculated sperm. This has led a number of clinicians world-wide to offer testicular sperm retrieval for ICSI in non-azoospermic males with high SDF. This practice has remained controversial due to lack of high quality evidence.

Keywords: DNA damage; DNA fragmentation; Infertility, male; Sperm retrieval

INTRODUCTION

In the era of intracytoplasmic sperm injection (ICSI), many barriers to fertilization have been overcome leading to a focus on the quality and development of the embryo. As part of this, the assessment of sperm quality parameters has become crucial. DNA damage in spermatozoa has been associated with reduced rates of *in vitro* fertilization, impaired development of the embryo into the blastocyst stage, increased rates of early pregnancy loss, and poor fertility outcomes following natural or assisted conception [1,2]. Sperm from the epididymis is more prone to DNA damage, due to the oxidative stress associated with epididymal transit as described by Esteves et al [3] who found a DNA fragmentation index (DFI) of 8.3% in testicular

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sperm *versus* 40.7% in ejaculated sperm. Greco et al [4] also reported significantly lower ($p<0.001$) DFI in the testes (4.8%±3.6%) compared with the ejaculated sperm samples from the same individuals (23.6%±5.1%).

Oxidative stress is an alteration of the body's reduction/oxidation potential that results from exaggerated levels of reactive oxygen species and/or reduction in the antioxidant defense system. It has been implicated in the pathophysiology of male infertility through multiple pathways including sperm lipid peroxidation, abortive apoptosis and DNA damage [5].

With this knowledge many clinicians are increasingly inclined to perform ICSI with testicular sperm in non-azoospermic patients who failed implantation and have high levels of DNA damage. However, this is not without controversy, since the use of testicular sperm involves surgical risks and a possible higher rate of aneuploidy. This article will review the current literature and evidence and discuss its support for this treatment strategy.

MATERIALS AND METHODS

The PubMed and Google Scholar databases were searched for articles published until March 2020 as per the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The search strategy was created by different combinations of the following entry terms: "DNA Damage"[Mesh], "DNA Fragmentation"[Mesh], "Infertility, Male"[Mesh], "Asthenozoospermia", "Azoospermia", "Oligospermia"[Mesh], "Teratozoospermia", "Sperm Retrieval"[Mesh], "Sperm aspiration", "Testicular Sperm", and "Assisted Reproduction". Manual search through references of the retrieved articles was performed as well. Studies evaluating the use of testicular sperm for ICSI in non-azoospermic men with high sperm DNA fragmentation (SDF) were included if assisted reproductive technology (ART) outcomes such as fertilization, pregnancy and miscarriage rates as well as live-birth rates were reported. Only English articles using human subjects were included. Non-comparative studies were excluded. To achieve an unbiased critical overview, meta-analysis and systematic review were not included in this first analysis. Conversely, they are critically discussed in later sections of this study. The quality of each included study was measured according to the system developed by the GRADE Working Group (Table 1).

RESULTS

The search described above resulted in 323 articles overall. These publications were screened by title and abstracts, resulting in 309 articles that failed to meet the inclusion criteria and were excluded. The remaining 14 studies were explored by the authors and 8 articles were included for critical analysis (Fig. 1).

Three of these studies were prospective in design, while three were retrospective studies and two were case-crossover studies. Various outcomes were evaluated. Live birth rate was reported by six studies [3,6-10], fertilization rate was evaluated by 7 studies [3,4,6-9,11], miscarriage was reported by 7 studies [3,6-11], and clinical pregnancy rate was reported by all eight studies. Table 1 describes the main characteristics of these studies [12].

DISCUSSION

1. Evidence in favor of testicular sperm

Several studies published, mainly in the last decade, assessed the use of testicular sperm in ICSI (T-ICSI) cycles of couples in which the male partner had a high SDF level [3,4,6-11]. In fact, five of these articles [3,6-9] report a significant increase in the live-birth rates after T-ICSI when compared to ICSI using ejaculated sperm (Ej-ICSI). A significant increase in clinical pregnancy rates has also been reported by five studies [4,7-9,11] and a significant decrease in miscarriage rate has been found in three of the articles [3,7,8] using testicular sperm. Four of these studies [3,4,6,11] were included in a meta-analysis study conducted by Esteves et al [13] in 2017 comparing reproductive outcomes of T-ICSI *versus* Ej-ICSI among men with high levels of DNA fragmentation in semen. The authors have analyzed fertilization rate, pregnancy rate, miscarriage rate and live birth rate in two subgroups: (a) patients with oligozoospermia and no previous ICSI attempts and (b) patients with normal sperm concentration and a previous failed ICSI with ejaculated sperm. This meta-analysis showed the benefit of T-ICSI regarding fertilization rates in patients with oligozoospermia and without a previous failed ICSI, but not in patients with normozoospermia and a previous failed ICSI. The analysis of clinical pregnancy rate favored testicular sperm in both scenarios as well as miscarriage and live birth rates. At first, this data may be considered enough to justify the

Table 1. Evidence on the use of testicular *vs*. ejaculated sperm

Study reference	Study population	Study design	Subject (n)	SDF assay	Fertilization rate	Pregnancy rate	Miscarriage rate	Live birth rate	Quality of evidence[a]
Greco et al [4] (2005)	- 2 previous ICSI failures - SDF >15%	Case-crossover study	T-ICSI: 18 Ej-ICSI: 18	TUNEL	T-ICSI: 74.9% Ej-ICSI: 70.8% (p>0.05)	T-ICSI: 5.6% Ej-ICSI: 44.4% (p<0.05)	NR	NR	+, very low
Esteves et al [3] (2015)	- Idiopathic oligozoospermia - SDF >30% - First ICSI cycle - Fresh sperm	Prospective study	T-ICSI: 81 Ej-ICSI: 91	Halo Sperm	T-ICSI: 56.1%±15.0% Ej-ICSI: 69.4%±17.0% (p=0.0001)	T-ICSI: 51.9% Ej-ICSI: 40.2% (p=0.13)	T-ICSI: 10% Ej-ICSI: 34.3% (p=0.012)	T-ICSI: 46.7% Ej-ICSI: 26.4% (p=0.007)	+++, moderate
Pabuccu et al [11] (2017)	- 2 previous ICSI failures - Normozoospermic - DFI>30%	Retrospective study	T-ICSI: 31 Ej-ICSI: 40	TUNEL	T-ICSI: 74.1±20.7 Ej-ICSI: 71.1±26.9 (p=0.619)	T-ICSI: 41.9% Ej-ICSI: 20% (p=0.045)	T-ICSI: 3.2% Ej-ICSI: 17.5% (p=NR)	NR	+, very low
Bradley et al [6] (2016)	- Non-ICSI failure - Fresh and frozen sperm	Retrospective study	- High SDF and no intervention (HN): 80 cycles - High SDF with intervention (HI): 368 cycles - Low SDF (LS): 1,727 cycles	SCIT	HN: 66.0% HI: 64.0% LS: 70.2% (HN *vs.* LS p<0.05) (HI *vs.* LS: p<0.001)	HN: 28.9% HI: 43.7% LS: 43.6% (p=NS)	HN: 11.4% HI: 11.6% LS: 11% (p=NS)	HN: 24.9% HI: 43.8% LS: 40.6% (HN *vs.* HI: p<0.05)	+, very low
Arafa et al [8] (2018)	- SDF>30% after treatment - Previous ICSI failure	Case-crossover study	T-ICSI: 36 Ej-ICSI: 36	Halo Sperm	T-ICSI: 47.8% Ej-ICSI: 46.4% (NS)	T-ICSI: 38.89% Ej-ICSI: 13.5% (p <0.0001)	T-ICSI (n): 0 Ej-ICSI (n): 2 (p<0.0001)	T-ICSI (n): 17 Ej-ICSI (n): 3 (p<0.0001)	++, low
Herrero et al [7] (2019)	- 2 previous ICSI failures - Fresh sperm	Prospective study	- T-ICSI and TUNEL: 50 - Ej-ICS and TUNEL: 46 - T-ICSI and SCSA: 52 - Ej-ICSI and SCSA: 44	SCSA/TUNEL	T-ICSI: 62.7% Ej-ICSI: 63.6% (NS)	T-ICSI: 27.9% Ej-ICSI: 10% (p<0.025)	T-ICSI: 25% Ej-ICSI: 41.7% (p<0.05)	Cumulative live birth rate: T-ICSI: 23.4% Ej-ICSI:11.4% (p<0.05)	+, low
Zhang et al [9] (2019)	- DFI>30% - Oligozoospermia or normozoospermia	Prospective study	T-ICSI: 61 Ej-ICSI: 41	SCSA	T-ICSI: 70.4% Ej-ICSI: 75% (NS)	T-ICSI: 36% Ej-ICSI: 14.6% (p=0.017)	T-ICSI: 0% Ej-ICSI: 3.3% (p=0.159)	T-ICSI: 41% Ej-ICSI: 9.8% (p=0.001)	++, low
Alharbi et al [10] (2019)	- At least 1 failed ICSI cycle - SDF 15%–30% and SDF >30% - Only fresh embryo transfer	Retrospective	- T-ICSI: 37 - Ej-ICSI: 56 (second ICSI, SDF unknown) - Ej-ICSI-high SDF (15%–30% or >30% - at least 1 failed ICSI): 31	SCSA	NR	T-ICSI: 48.6% Ej-ICSI: 48.2% Ej-ICSI-high SDF: 38.7% (NS for both)	T-ICSI: 11.1% Ej-ICSI: 11.1% Ej-ICSI-high SDF: 38.7% (NS for both)	T-ICSI: 36.4% Ej-ICSI: 33.3% Ej-ICSI-high SDF: 30% (NS for both)	+, very low

SDF: sperm DNA fragmentation, ICSI: intracytoplasmic sperm injection, DFI: DNA fragmentation index, T-ICSI: ICSI using testicular sperm, Ej-ICSI: ICSI using ejaculated sperm, TUNEL: terminal deoxynucleotidyl transferase dUTP nick end labeling, SCSA: sperm chromatin structure assay, SCIT: sperm chromatin integrity test, NS: not significant, NR: not reported.
[a]Extract from Guyatt et al's guideline [12].

use of testicular sperm in these patients to achieve better ICSI outcomes. However, this is not without controversy as these studies have several weaknesses (Fig. 2).

2. Critical analysis of study designs

The quality of the aforementioned evidence is low due to a gamut of limitations noted in these articles. Although live-birth rate is acknowledged as the most important outcome in assisted reproduction studies, it is not reported by two of the eight studies [4,11]. The study design of two of them [4,8] is classified as case-crossover study, meaning that the same individuals are analyzed in different time points and each subject serves as his own control. This type of study is considered methodologically fragile and it should not be used to draw conclusions on ART outcomes [14]. Further, Bradley et al [6] used both frozen and fresh samples for ICSI and the distribution of each in both groups (T-ICSI and Ej-ICSI) is not described, therefore the results are difficult to interpret. Additionally, in spite of using testicular sperm with less SDF, there are considerable shortcomings regarding the DNA fragmentation testing in each study: (i) SDF was not even measured in testicular sperm in three studies [6,9,11]; (ii) SDF values in each group were not reported in two studies [8,9]; and (iii) SDF cut-off values used by Greco et al [4] as well as Herrero et al [7] are controversial and the last study shows possible selection bias regarding SDF values. Moreover, in order to properly evaluate the effectiveness of any male infertility treatment, female factors need to be controlled for. Female body mass index, FSH levels, anti-müllerian hormone levels, endometrial thickness, and antral follicle count was either not reported or heterogeneously matched by several of these studies [3,7,11]. Besides these limitations, considering these studies in general, they are grossly incomparable, since they have different study designs (prospective,

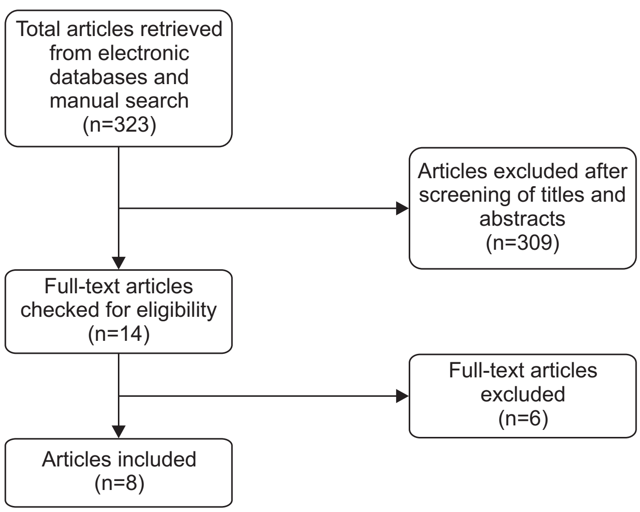

Fig. 1. Flow diagram of literature review and study selection.

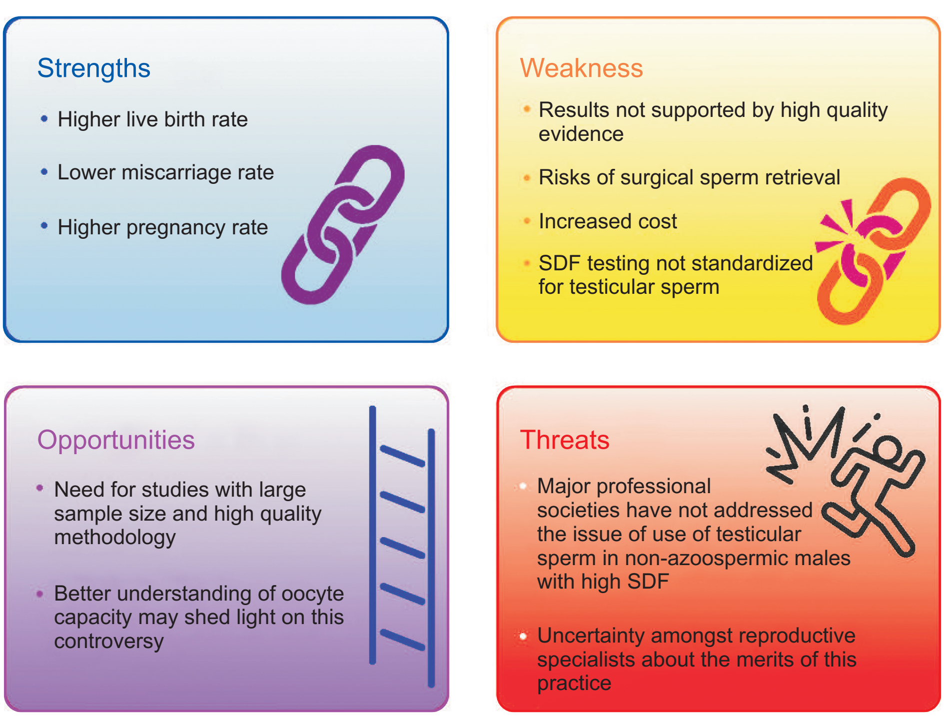

Fig. 2. SWOT (Strengths/Weakness/Opportunities/Threats) analysis. SDF: sperm DNA fragmentation.

retrospective, crossover); different and heterogeneous study populations with regards to their seminal status (severe oligozoospermia, moderate oligozoospermia, mild oligozoospermia and even normozoospermia), number of previous failed ICSI cycles, and protocol used for ovarian stimulation and SDF test used. With respect to the meta-analysis conducted by Esteves et al [13], despite the efforts to separate patients into subgroups according to semen parameters and previous IVF/ICSI cycles, the population in each study was heterogeneous and the studies included have major limitations, weakening its conclusions.

3. Disadvantages of surgical sperm retrieval

Testicular sperm retrieval in non-azoospermic males is commonly harvested by testicular sperm aspiration (TESA), testicular sperm extraction (TESE) or microscopic TESE (microTESE). Therefore, it is important to keep in mind the fact that using testicular sperm involves a surgical procedure which in and of itself can have complications. This includes testicular hematoma, wound infection, postoperative pain, total testicular loss, and anesthetic complications. Together with the high cost of the procedure which is often times not covered by insurance, testicular sperm harvesting should not be considered a benign procedure. None of the comparative studies retrieved in this review addressed this issue and only one study reported complication rates [3]. Further, there is concern about the possibility of an increased aneuploidy rate in testicular sperm. Moskovtsev et al [15] showed that testicular sperm have 2–3 fold higher aneuploidy rates than ejaculated samples (12.41%±3.7% *versus* 5.77%±1.2%, $p<0.05$). These results have been contested, though, in a recent study conducted by Cheung et al [16] who demonstrated safe utilization of testicular sperm, in regard to aneuploidy. Yet, it is important to highlight that the studies concerning aneuploidy have small samples and are inconclusive.

4. Limitation of sperm DNA damage evaluation techniques

There is large variability among the tests used to determine DNA damage. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), Comet (single cell gel electrophoresis), sperm chromatin structure assay (SCSA) and sperm chromatin dispersion (SCD) are all used clinically. TUNEL and Comet are direct assays that measure DNA fragmentation, while SCSA is an indirect assay and SCD evaluates chromatin maturity. The lack of standardized protocols and different measurements result in large variability among different laboratories, including ones that even use the same tests [17]. Additionally, testicular sperm differs from ejaculated sperm in their DNA and surface markers, as well as remodeling of histone/protamine complex which further complicates matters [18-20]. Because of these differences, SDF assessment has yet to be standardized in testicular sperm [21].

5. Evidence against the use of testicular sperm

A meta-analysis conducted by Abhyankar et al [22] included 5 cohort studies, comprising 272 ICSI cycles and 2,547 injected oocytes, using testicular and ejaculated sperm from men with cryptozoospermia, or semen that had to undergo repeated centrifugation to locate sperm. Centrifugation has been shown to increase the production of reactive oxygen species [9]. Despite this, the authors showed no difference in fertilization or pregnancy rates with ICSI, when comparing testicular and ejaculated sperm in men with cryptozoospermia [22]. This meta-analysis has some important limitations as well, such as the variability of the definition of cryptozoospermia among the selected studies and the fact that two of these studies used both fresh and frozen sperm samples [23,24].

In 2019, Alharbi et al [10] conducted a retrospective, comparative analysis on the use of testicular sperm harvested by TESA in 37 non-azoospermic males and compared them with the results using ejaculated sperm in a cohort of 31 men in the same clinic, all with SDF>15%, assessed by SCSA. Both groups had at least one previous failed ICSI cycle and were divided into two groups (SDF>15% and SDF>30%). They failed to report any significant improvement with testicular sperm independent of SDF level in clinical pregnancy rates per embryo transfer, miscarriage rate and live birth rate. This study also has limitations, such as the retrospective design and the fact that the ejaculated sperm group had significantly higher sperm concentration and sperm motility as well as lower DFI than the T-ICSI group.

Awaga et al [25] conducted a systematic review evaluating ICSI outcomes using fresh ejaculated spermatozoa *versus* surgically extracted spermatozoa from the testes in patients with abnormal semen parameters but without azoospermia. Case reports, case-crossover studies or studies using frozen spermatozoa were not included. Of the 4 studies that met this criteria, only 2 articles, Esteves et al [3] and Pabuccu et al [11], included patients with high DNA fragmentation. This study concluded that a meta-analysis was not possible since each study used different populations, ovarian stimulation protocols and SDF assays, emphasizing the lack of adequate data to support performing an invasive procedure in non-azoospermic men [25].

6. Limitation of the current review

The quality of a systematic review is only as good as the studies themselves. In this case, the majority of the studies were of low quality. Three of the included studies were retrospective and the remaining 5 were prospective observational or cross-over studies. Further research, including randomized and blinded studies are required to reach a firm understanding of the benefits of using testicular sperm in patients with repeated ICSI failure. Moreover, the heterogenous nature of the included studies prevented us from performing quantitative analysis which is another limitation of this review.

CONCLUSIONS

The belief that SDF contributes to unsuccessful ICSI in some cases has led to the idea of using testicular sperm with lower SDF for ICSI cycles in non-azoospermic men in the hopes of achieving a successful pregnancy. Several investigators have published their results showing the benefit of this technique [3,6-10,13,22]. However, it is important to look at these studies critically and have a broad understanding of the complex mechanisms of sperm DNA damage [26]. While oxidative stress induced DNA damage that primarily occurs during sperm maturation and transit through the epididymis is believed to be the most common etiology, intratesticular alterations in chromatin remodeling can also co-exist resulting in testicular retrieval of sperm with fragmented DNA. Therefore, the adequate clinical management of patients with high SDF has to be considered as first line therapy, rather than used as a justification to pursue a potentially harmful surgical sperm retrieval. The control of exogenous factors such medication use, obesity and smoking combined with an increase of ejaculation frequency and use of appropriate antioxidants can help reduce DNA fragmentation and may decrease the need for invasive procedures. The use of adequate sperm selection methods may also provide sperm with lower SDF levels [27,28].

The use of testicular sperm may seem like a reasonable alternative to achieve a sample with a lower DNA fragmentation [15]. However, the possibility of higher aneuploidy rates and the fact that DNA fragmentation tests are not standardized in testicular sperm should be carefully considered. In addition, the mechanism of intratesticular DNA damage and its interactions with extratesticular pathways of DNA damage in each patient is unclear.

Results from several meta-analyses have suggested that while SDF has little or no impact on ICSI pregnancy rate, it is associated with a significant increase in the miscarriage rate following ICSI with an odds ratio between 2.1 and 2.5 [2,29,30]. Nonetheless, this association has been mostly extracted from retrospective studies of heterogenous design and using different SDF assays [30], making interpretation of the data and broad applicability difficult. In a recently published clinical guideline, endorsed by the Society of Translational Medicine, we recommended the using testicular sperm in patients with a history of recurrent miscarriages following ICSI, defined by two or more miscarriages occurring with Ej-ICSI, in the context of high SDF [31]. It is crucial, nonetheless, to remember that this approach is advised only after adequate patient counselling and once all efforts at lowering SDF have been tried.

It is important to emphasize that the majority of articles published on the use of testicular sperm in patients with high SDF consist of small cohorts or case series, comparing different patient populations. Additionally, several of these studies lack adequate control groups, a proper evaluation of possible female factors, and more importantly some do not report live birth rates. These studies also do not take into account the higher costs and risks involved in harvesting and using testicular sperm.

Despite recent publications advocating the use of testicular sperm in non-azoospermic men with repeated failed ICSI cycles and high DNA fragmentation, the majority of studies used for this claim are of poor quality and high heterogeneity, weakening the level of evidence in support of this approach. Studies using more rigorous study design, control groups, and appropriate outcomes are needed to address the drawbacks of the current literature and more definitively determine if testicular sperm should be used for non-azoospermic patients who have failed previous ICSI.

TAKE-AWAY MESSAGE

SDF testing has not been validated for testicular sperm making interpretation of testicular SDF levels difficult.

Some studies report a positive impact on ART outcomes using testicular sperm, however the quality of evidence is weak.

New well designed studies are warranted to make a definitive conclusion on the use of testicular sperm in these cases.

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Conflict of Interest

The authors have nothing to disclose.

Author Contribution

Conceptualization: RFA, AA. Literature review and formal analysis: RFA. Writing – original draft: RFA. Writing – reviews & editing: AA, RFA, AM, SV, NNT, CLC, NP, EBJ, SG.

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