# High Sperm DNA Damage
## Does Testicular Sperm Make Sense?

Keith Jarvi, MD, FRCSC

**KEYWORDS**

• Sperm DNA integrity • In vitro fertilization • Sperm selection • Prospective cohort study
• Live birth rates • Pregnancy rates • Testicular sperm retrieval

**KEY POINTS**

- High sperm DNA damage increases the risks of pregnancy loss.
- Testicular sperm have less DNA damage than sperm in the ejaculate.
- Using testicular sperm for in vitro fertilization for men with high levels of sperm DNA damage is widely used.
- Presently, there is insufficient evidence to conclude that the use of testicular sperm increases live birth rates compared with ejaculated sperm for men with high levels of sperm DNA damage.
- The use of testicular sperm retrieval for in vitro fertilization to manage men with high sperm DNA damage is not supported by the literature published to date.

## BACKGROUND

In North America, approximately 15% of couples suffer from subfertility. In approximately 35% of these couples, a male factor is identified for the subfertility.[1] There are a variety of treatments available for men with subfertility, but quite often in vitro fertilization (IVF) or intra-cytoplasmic sperm insertion (ICSI) is used to help couples with male factor infertility. IVF is the process in which sperm are incubated with oocytes, whereas ICSI is the process in which individual sperm are injected directly into oocytes. Because ICSI requires the use of very few sperm and because the injection of the sperm directly into the oocyte ICSI means that the sperm do not require the ability to bind to or penetrate the oocyte, ICSI is now widely used to treat men with abnormal semen parameters, including men with low sperm counts and sperm motility, as well as for men with low numbers of morphologically normal sperm. ICSI has allowed millions of couples with infertility to become biological parents.

In North America, more than 200,000 IVF or ICSI cycles are performed yearly.[2] In Europe, there were more than 686,000 cycles performed in 2013, and in Japan, 244,000 cycles in 2015.[3,4] If a male factor is identified, more than 90% of the cycles are ICSI rather than IVF alone.[2]

The medical costs of IVF can be quite high but vary substantially by country. Chambers and colleagues[5] reported that the cost of an IVF cycle (in 2006 US$) ranged from $12,513 in the United States to $3956 in Japan. In addition, a significant amount of time and effort is taken by the couples undergoing the fertility treatments, with multiple visits to the infertility units for investigations and treatments.

Reported first in 1992 by Palermo and colleagues,[6] the use of ICSI has certainly revolutionized the treatment of male factor infertility. This group reported that ICSI bypassed the natural selection process, allowing for high fertilization, pregnancy, and live birth rates for couples with infertility, with the success rates being independent of the sperm count, motility, or morphology.[7] Other groups subsequently reported clinical studies that ICSI fertilization and pregnancy rates

Division of Urology, Department of Surgery, Institute of Medical Science, University of Toronto, Lunenfeld-Tannenbaum Research Institute, Mount Sinai Hospital, 60 Murray Street, 6th Floor, Box 19, Toronto, Ontario M5T 3L9, Canada
*E-mail address:* keith.jarvi@sinaihealth.ca

Urol Clin N Am 47 (2020) 165–174
https://doi.org/10.1016/j.ucl.2019.12.009

were not affected by sperm counts, motility, morphology, or even the source of the sperm (ejaculated vs epididymal) as long as the sperm were alive.[8–10]

This optimism that ICSI outcomes were independent of sperm parameters has been challenged by more recent studies showing reduced pregnancy rates and live birth rates for couples with male factor infertility. Strassburger and colleagues[11] in 2000 reported lower fertilization and pregnancy rates as well as lower live birth rates if the men had lower sperm counts, and De Vos and colleagues[12] in 2003 noted higher pregnancy rates when morphologically normal sperm were used for ICSI.

Although routine semen testing measures the sperm count, motility, and morphology, other tests of sperm function/capacity have been developed: some of the most widely used approaches are assays to measure the sperm DNA integrity (**Fig. 1**). The most common tests in use are the sperm chromatin structure assay, the sperm chromatin dispersion test, the COMET assay (**Fig. 2**), and the TUNEL assay (terminal deoxynucleotidyl transferase dUTP nick end-labeling assay).[13] Unfortunately, there is no standardized approach to the measure of sperm DNA damage, and reports on sperm DNA damage are typically a numeric value that is then interpreted as a binomial result (either high or normal/low levels of DNA fragmentation). With such a variety of different measures and normal reported ranges of sperm DNA damage, it is difficult to interpret different studies using sperm DNA damage levels as a metric.

Sperm DNA damage may occur during spermatogenesis with the induction of apoptosis (reviewed by Sakkas and Alvarez[14] in 2010), during spermiogenesis[15] (during chromatin remodeling), or posttesticular with damage owing to reactive oxygen species,[16,17] reduced seminal antioxidants,[18] and exposure to environmental/lifestyle factors, such as pollution, cigarette smoking, chemotherapies, advanced age, and some drugs.[19–21]

## *Impact of High Sperm DNA Damage on Intracytoplasmic Sperm Insertion Outcomes*

There is now quite compelling evidence that elevated rates of sperm DNA fragmentation are associated with compromised ICSI outcomes. In a metaanalysis in 2008, Collins and colleagues[22] found an association between sperm DNA fragmentation as measured by standard sperm DNA integrity assays and pregnancy rate (odds ratio [OR] 1.44: 95% confidence interval [CI] 1.03–2.03) with ICSI, whereas Zini and colleagues[23] in 2008 noted that sperm DNA fragmentation was predictive of pregnancy loss following IVF/ICSI cycles (OR 2.48: 95% CI 1.43–5.2). Zhao and colleagues[24] in 2014 in an updated metaanalysis confirmed the above findings and noted that abnormally high levels of sperm DNA fragmentation was associated with higher pregnancy loss rates following IVF/ICSI (OR 2.68: 95% CI 1.40–5.14).

### *How frequently is high sperm DNA fragmentation found?*

There are several studies reporting on the frequency of high sperm DNA fragmentation with reported rates up to 40% of infertile men,[25] but the author's series from Toronto found the rates of high sperm DNA fragmentation depended on the diagnosis, with high sperm DNA fragmentation found in 48% of men with bacteriospermia, 30% of men with varicoceles, and 22% of the men with idiopathic infertility.[26] Only 8% of fertile men in the series by Zini and colleagues[27] were found to have significant sperm DNA fragmentation.

### *How to treat men to reduce sperm DNA fragmentation?*

There are potentially reversible causes for high sperm DNA fragmentation, including varicoceles, infections, and smoking.[28] A recent metaanalysis showed that varicocelectomy reduces sperm DNA fragmentation rates by −3.37% (95% CI: −4.09 to −2.65).[29] Smoking has long been associated with male infertility, but the negative impact of smoking on sperm DNA fragmentation has only more recently been reported.[30–32] Although smoking cessation is recommended, the impact of cessation on sperm DNA has not been reported. Infections and inflammations of the male reproductive tract may also be related to sperm DNA fragmentation, with improvements in DNA integrity with specific therapies.[33–35] For most men, no potentially reversible causes are identified. These men have been treated with antioxidants with evidence of a significant reduction of sperm DNA fragmentation (reviewed by Zini and colleagues[36] and Showell and colleagues[37]) in 1 metaanalysis of 2 studies showing a reduction of −13.85% (95% CI: −17.85 to −10.41). Despite these therapies, many men end up with abnormally high rates of sperm DNA fragmentation, which may be contributing to IVF/ICSI failures.

### *If therapies to improve sperm DNA integrity are ineffective, are there alternative ways to improve the reproductive outcomes with intracytoplasmic sperm insertion for men with sperm DNA fragmentation?*

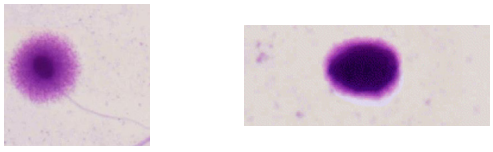
It is well recognized that there is intense sperm-to-sperm variability (within the same semen specimens) in the levels of DNA fragmentation.

**Sperm Chromatin Structure Assay**

Following mild acid denaturation of sperm DNA acridine orange binds to double stranded DNA (non-denatured = green) or single stranded (denatured =red)

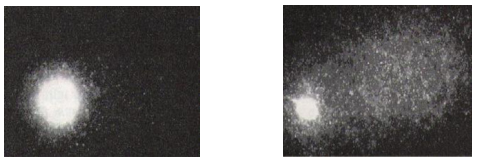

**Sperm Chromatin Dispersion assay**

Sperm with intact DNA = loops around sperm. Sperm with damaged DNA = no loops around sperm.




**Comet assay**

Electrophoretic current moves the fragmented DNA into the tail of the comet




**TUNEL Assay**

Fluorescent nucleotides dUTP are incorporated into single or double stranded DNA breaks. The fluorescent signal increases with the number of strand breaks.

**Fig. 1.** Commonly used assays to measure sperm DNA damage levels. dsDNA, double-stranded dsDNA; ssDNA, single-stranded DNA; TUNEL, terminal deoxynucleotidyl transferase nick end-labeling assay.

Conceptually, if there was some way to choose individual sperm with less DNA fragmentation, this method should improve the reproductive outcomes following ICSI. Unfortunately, with the present technology in use, there is no way to measure the level of DNA fragmentation in an individual sperm and then use that sperm for ICSI.

## *Selection of Sperm with Less DNA Damage*

The standard sperm selection techniques use a combination of centrifugation of the sperm through a density gradient, often followed by a "swim-up" procedure to select highly motile sperm.[38] This process does select sperm with higher motility and with lower DNA fragmentation.[39] This technique remains the standard selection technique for most fertility centers in North America.

There have been several other different procedures used to select sperm with lower DNA fragmentation. *Hyaluronan binding* has been used to select sperm with lower DNA fragmentation rates: these sperm have subsequently been used for the ICSI procedure (PICSI technique: physiologic hyaluronan-selected intracytoplasmic sperm injection).[40] This technique has not been shown to improve live birth rates, with a very large recent study recommending that PICSI be abandoned for sperm selection.[40,41] Others have reported the use of high-resolution imaging of the sperm

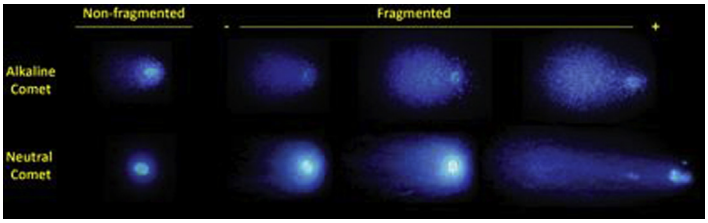



**Fig. 2.** Examples of Comet assays showing "high," "normal," and low sperm DNA integrity. An electrophoretic current separates the fragmented DNA into the tail of the comet, leaving the nonfragmented DNA in the head of the comet.

to select sperm with normal sperm organelles (motile sperm organelle morphology examination) and intracytoplasmic morphologically selected sperm injection (IMSI), both of which select sperm with lower DNA fragmentation but neither of which have been shown to increase live birth rates with the exception of the potential use for recurrent implantation failures for the IMSI procedure.[42]

Recently, there has been a significant research effort to develop microfluidics devices to select sperm with lower DNA fragmentation for IVF/ICSI.[43–50] Most of these devices use some type of microchannel, which separates sperm based on the ability to negotiate/swim through the microchannel (**Table 1** lists available devices). The devices avoid centrifugation, which was previously shown to increase DNA fragmentation rates.[39] Consistently, these devices are able to select highly motile sperm with low DNA fragmentation, outperforming the standard sperm selection technique in selecting sperm with high DNA integrity (reviewed by Nosrati and colleagues[47]).[44,47,51] For example, Quinn and colleagues[44] in 2018 reported that sperm selected using a microfluidic device had virtually undetectable rates of sperm DNA fragmentation as measured by the sperm chromatin dispersion assay, rates that were significantly less than the rates in sperm selected using a standard density gradient and swim-up sperm selection technique. Unfortunately, there are no data for any microfluidic device on aneuploidy rates for selected sperm. Although the microfluidic approaches look promising for the selection of sperm for IVF/ICSI, *there are no randomized controlled trials documenting improvements in reproductive outcomes using these devices*. In 2018, Yetkinel and colleagues[43] reported on a randomized controlled trial on the use of the Fertile Chip device to select sperm for ICSI outcomes compared with the standard sperm preparation technique. They reported similar fertilization, pregnancy, and live birth rates between the 2 groups. This group selected couples with unexplained infertility not based on any particular semen parameter. There is a registered clinical trial on the use of the Zymot device for couples who have had "poor embryo quality" in a previous IVF cycle, but the details of this study were not recorded at clinicaltrials.gov.

Despite the lack of well-controlled studies documenting higher live birth rates using microfluidics-selected sperm, several companies are selling these devices for use to select sperm before IVF, and 1 device (Zymot or FERTILE) is approved by the Food and Drug Administration for sale in the United States. The Zymot device is commercially available and in clinical use to select sperm for ICSI in North America (according to Koek Biotech, having been used for thousands of ICSI cycles).

### *Testicular Sperm Retrieval*

Some centers are now offering testicular sperm retrieval (TSR) for men with high sperm DNA fragmentation. The rationale for offering TSR for men with high sperm DNA fragmentation undergoing ICSI is the finding that ejaculated sperm have significantly higher rates of sperm DNA fragmentation than testicular sperm, with a metaanalysis by Esteves and colleagues[52] showing a significantly lower sperm DNA fragmentation rate in testicular versus ejaculated sperm (8.9% $\pm$ 5.1% vs 33.4% $\pm$ 12.8%).[53,54]

Presently, none of the major fertility associations (American Society of Reproductive Medicine, European Society of Human Reproductive Endocrinology, and the Canadian Fertility Andrology Society) supports the use of TSR to treat men with high sperm DNA damage. In fact, the American Society of Reproductive Medicine does not even support the routine use of sperm DNA integrity testing.[55] Despite the lack of support from the major fertility associations, this procedure is now being offered in an unknown number of clinics throughout the world. In a recent survey of Canadian fertility clinics, 70% were performing TSR with ICSI for nonazoospermic men (reported by Zini and colleagues[56]).

### *Comparing Testicular Sperm Retrieval Versus Ejaculated Sperm Reproductive Outcomes with Intracytoplasmic Sperm Insertion*

Although the evidence that sperm DNA damage is less in the TSR sperm than in the ejaculate is compelling, what evidence exists to support the use of TSR to improve live birth rates for men with high sperm DNA damage using ICSI? Greco and colleagues[54] were the first to report higher pregnancy rates in testicular versus ejaculated sperm for those with high sperm DNA fragmentation, with the reported pregnancy rate of 44% in the TSR group versus 6% in the ejaculated sperm group. Over the years, there has been a series of other noncontrolled studies showing improved pregnancy rates with TSR compared with ejaculated sperm for men with high sperm DNA damage.[57–64] A study by Alharbi and colleagues[65] did not identify increased live birth rates.

In the metaanalysis by Esteves and colleagues[52] reported in 2017, it was found that ICSI using testicular sperm compared with ejaculated sperm resulted in lower fertilization rates (59.8% vs 68.7%, $P<.001$), higher clinical pregnancies (50% vs 29.4%, $P<.001$), and higher live birth rates

**Table 1**
**Microfluidic devices being marketed**

| Device | Selection Mode | % DNA Fragmentation Index | | Concentration (Selected/Raw) | Clinical (IVF/ICSI) Trial/Countries | IVF/ICSI Test Results | Reference/Web Site |
|---|---|---|---|---|---|---|---|
| | | Raw | Selected | | | | |
| FERTILE | 3D swimming | 31–40 | 0–0.2 | 0.28 | In progress/ United States, Turkey | • Pregnancy %: 34% when chip is used vs 23% with Percoll method (performed in Turkey) | • Quinn et al, 2018[72]<br>• http://www.koekbiotech.com |
| QUALIS | 3D swimming | N/A | 0–9 | 0.08 | Japan | • No information available | • cho et al, 2003[73]<br>• http://www.menicon-lifescience.com |
| ZECH SELECTOR  | 3D swimming | 5–42.1 | 0–2.5 | N/A | No information available | • No information available | • Seiringer et al, 2013[74]<br>• https//www.kinderwunsuch.at/de/zech-selector.com |
| CS10 | Electrophoretic separation | 16 | 5 | 0.18 | Australia | • No significant difference in fertilization compared with density-gradient centrifugation (62.4% vs 63.6%) | • Fleming et al, 2008[75]<br>• http://www.memphasys.com.au/ |
| Seaforia  | Thermotaxis+ 3D swimming | N/A | N/A | 0.20 | Australia | • No information available | • Irving et al, 2013[76]<br>• http://wwwlotusbio.com |

*Abbreviation:* N/A, not applicable.

(46.9% vs 25.6%, $P<.001$) with an OR of 2.58 (95% CI: 1.54–4.35). However, the study was limited with the included reports being noncontrolled, in some cases comparing ICSI outcomes sequentially for the same couple and only a total of 2 studies reported live birth rates. As previously reported by Khan and colleagues[66] in 1996, these types of crossover studies lead to significant overestimation of pregnancy rates. In addition, data used in the metaanalysis included a study in which the sperm had been cryopreserved without a subanalysis of the effect of cryopreservation on the reproductive outcomes.[63]

Awaga and colleagues[67] in 2018 performed a systematic review on the use of testicular sperm for men without azoospermia and identified a total of 4 studies eligible for the review, with only 2 studies specifically on the use of TSR for high sperm DNA damage. Because of the population heterogeneity, a metaanalysis was not feasible, so the investigators performed a systematic review. The invstigators' conclusion was that the existing studies were too heterogeneous to compare and that the data did not support the use of TSR to manage men with high sperm DNA damage.

### *What Are the Risks Associated with Testicular Sperm Retrieval?*

There are risks associated with the biopsy and even potentially the use of testicular sperm. Esteves and colleagues[61] reported a surgical complication rate of 6.2%, although this included no significant complications. In the author's unpublished series (Jarvi and Lo, 2014) following 50 men after a testis biopsy, there were no significant complications and only 1/50 had an intratesticular hematoma documented by ultrasound that resolved spontaneously. The author's series also has evidence that testicular sperm aneuploidy rates are significantly higher than ejaculated sperm aneuploidy rates. Moskovtsev and colleagues[68] reported that although sperm DNA fragmentation rates were lower in testicular versus ejaculated sperm (14.9% $\pm$ 5.0% vs 40.6% $\pm$ 14.8%, $P<.05$), the aneuploidy rates for 5 analyzed chromosomes using fluorescent in situ hybridization were significantly higher in the testicular sperm (12.41% $\pm$ 3.7% vs 5.77% $\pm$ 1.2%, $P<.05$). The group suggested that the apparent advantage of lower sperm DNA damage in the testicular sperm may be offset by the disadvantage of higher aneuploidy rates. However, Cheung and colleagues[64] in 2019 reviewed their results prospectively comparing sperm DNA aneuploidy rates measured by whole-exome sequencing in ejaculated versus testicular sperm, finding that the testicular sperm did not have significantly different aneuploidy rates.

### *What Are the Potential Cost Advantages of Testicular Sperm Retrieval for Men with High Sperm DNA Damage?*

For this calculation, assume that the metaanalysis of Esteves and colleagues[52] provides accurate estimates of the effect size of TSR on live birth rates; then, a cost/live birth can be calculated (biopsy costs $1250–$2500 in Canada based on fees paid in Montreal and Toronto, IVF cost US$ $12,513 in 2006; **Table 2**).

For individual patients and payers, this is a potential significant saving. Considering that 22% of men with idiopathic infertility have high rates of sperm DNA damage, if TSR actually results in higher pregnancy rates, there would be a significant impact on payers if TSR was adopted.

### *What Is Required to Further Study the Role of Testicular Sperm Retrieval in the Management of Men with High Sperm DNA Damage?*

Presently, the reports available do not provide adequate evidence to support the use of TSR to manage men with high sperm DNA damage. However, the relatively low risk associated with the TSR procedure, the available studies that suggest a possible positive effect on live birth rates, and the lack of a viable proven alternative to improve pregnancy rates for men with high sperm DNA damage all lead to the obvious conclusion, that further studies are needed in the area before concluding that TSR should be a standard approach to manage men with high levels of sperm DNA damage.

There are several issues that need to be addressed: a lack of a standard technique to

**Table 2**
**Cost per live birth for testicular versus ejaculated sperm used for intracytoplasmic sperm insertion for men with high sperm DNA damage**

| | Biopsy, $ | IVF Direct Cost, $ | Live Birth Rate | Cost/Live Birth, $ | Saving/Live Birth, $ |
|---|---|---|---|---|---|
| Ejaculated sperm | — | 12,513 | 0.256 | 48,878 | — |
| Testicular sperm | 1875 | 12,513 | 0.469 | 30,678 | 18,200 |

measure sperm DNA damage levels and a lack of standard normal range values limit the ability to compare studies of sperm DNA damage.[13] Second, the standard reports on sperm DNA damage are categorical (reported as either high or normal/low), whereas the numerical value does provide added information on prognosis. Although a cutoff value for DNA damage is useful to provide guidance on the choice of assisted reproductive technologies (Spano and colleagues[69] using a cutoff value to predict the need for IVF or ICSI), it has become clear that very high rates of sperm DNA damage have different ICSI success rates than those with marginally elevated rates of sperm DNA damage.[70]

Complicating this, a controlled trial to definitively answer the question about the role of TSR for men with high sperm DNA damage would be ideal, but difficult, because recruitment would be challenging.[71]

## SUMMARY

High levels of sperm DNA fragmentation lead to poorer reproductive outcomes, with lower pregnancy and live birth rates following IVF and ICSI. There is active research on techniques to select sperm from the semen with the least DNA damage, but presently, none of the techniques has been proven to increase live birth rates. An alternative has been to retrieve sperm from the testicle. The sperm in the testicles has not been exposed to the more hostile environment of the epididymis/vas deferens and has less DNA damage than ejaculated sperm. Many fertility centers offer this sperm retrieval procedure for men with high ejaculated sperm DNA damage, despite this procedure not being supported by the major fertility organizations. The existing studies on the use of sperm retrieval in this setting are single-center, prospective, noncontrolled studies and do not provide adequate evidence to support the use of sperm retrieval to treat men with high levels of ejaculated sperm DNA damage. Further studies are required before the acceptance of TSR for high sperm DNA damage as a standard of care.

## DISCLOSURE

The authors have nothing to disclose. There is no funding to support this research.

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