Use of testicular sperm for ICSI in oligozoospermic couples: how far should we go?

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ABSTRACT: In 1992 and subsequently, several reports indicated that ICSI was a successful technique to achieve clinical pregnancy and live birth using spermatozoa with severely impaired characteristics. The initial optimism over the ability of ICSI to overcome significant sperm abnormalities was later tempered by the findings of more recent publications suggesting that some sperm deficits may not be as effectively treated with ICSI. In search for effective treatment for couples with severe male factor, a number of small retrospective and prospective studies have reported high pregnancy and live birth rates using testicular sperm for men with necrozoospermia, cryptozoospermia and oligozoospermia with or without elevated sperm DNA damage. Although the data suggest that there may be some benefit in performing testicular sperm retrieval (TSR)-ICSI in select groups of non-azoospermic fertile men, there are potential risks involved with TSR. Clinicians should balance these risks prior to the recommendation of TSR-ICSI on the result of a semen analysis or sperm DNA test alone. Careful evaluation and management of male factor infertility is important. The use of TSR-ICSI in the absence of specific sperm DNA defects is still experimental.

Key words: testicular sperm / sperm retrieval / ICSI / male infertility / sperm DNA

Early experience with ICSI: encouraging results for all male factor infertility

Ever since Palermo et al. described it in 1992, ICSI has become a critical tool in the armamentarium used to manage male infertility (Palermo et al., 1992). The seminal paper’s claims that ICSI bypasses the natural selection process in fertilization and enables the successful use of spermatozoa with severely impaired characteristics to achieve clinical pregnancy and live birth revolutionized the treatment paradigm for male infertility. In fact, Palermo reported that neither sperm concentration, morphology nor progressive motility had any impact on ICSI results (Palermo et al., 1993). Subsequently, several case series were published, reporting successful pregnancies with the use of severely impaired sperm for ICSI, serving to substantiate Palermo’s initial claims. In his retrospective case series of 130 couples with male infertility and previous failed IVF then undergoing ICSI, Mansour et al. (1995) showed no statistically significant differences in fertilization or pregnancy rates for men with <95% or >95% teratozoospermia. In a similar fashion, Nagy et al. (1995) reported that after assessing 966 ICSI cycles, neither oligozoospermia, teratozoospermia, asthenozoospermia nor severe oligoasthenoteratozoospermia (OAT) had an impact on fertilization or pregnancy rates. He found that the only sperm characteristic that portended a negative ICSI outcome was the injection of a totally immotile (and presumably dead) spermatozoon; this finding was also replicated by other series (Nagy et al., 1995; Nijs et al., 1996; Vandervorst et al., 1997). Taken together, the early evidence from numerous series suggested that except for couples with only absolutely immotile sperm available for injection, ICSI could successfully overcome even the most severe sperm characteristics.

Later experience with ICSI: poor outcomes in severely oligozoospermic couples

The initial optimism over the ability of ICSI to overcome significant sperm abnormalities has been tempered by the findings of more recent
Publications. Following the early reports of poor ICSI outcomes with totally immotile spermatozoa, Mitchell et al. (2006) examined a series of 21 patients with ultrastructural flagellar abnormalities causing asthenozoospermia and reported significantly lower clinical pregnancy rates in couples with sperm motility <5% compared with those with higher sperm motility (22% vs 84%, \( P = 0.04 \)) for couples with sperm motility <5% and >5%, respectively. Fertilization and implantation rates were also lower for men with asthenozoospermia, but the differences did not reach statistical significance (Mitchell et al., 2006).

While earlier studies concluded that teratozoospermia had little impact on ICSI outcomes, these studies failed to take into account the morphology of the spermatozoa used for injection into the oocytes. De Vos et al. (2003) reported significantly higher clinical pregnancy (37% vs 20%, respectively, \( P = 0.018 \)), implantation (32% vs 23%, respectively, \( P = 0.013 \)) and live birth (28% vs 20%, respectively, \( P = 0.006 \)) rates with the use of morphologically normal vs morphologically abnormal sperm for ICSI. However, other investigators have found limited, if any, effects of normal sperm morphology on ICSI outcomes (van den Hoven et al., 2015).

The initial promise regarding the ability of ICSI to overcome oligozoospermia has also been questioned by recent studies. Strassburger et al. retrospectively reviewed 1076 unselected ICSI cycles, stratified them according to sperm count and assessed fertilization, clinical pregnancy and miscarriage rates. When compared with couples with sperm concentration between \( 1 \times 10^5 \) sperm/ml and \( 1 \times 10^6 \) sperm/ml, cryptozoospermic couples had significantly lower fertilization and clinical pregnancy rates (46% vs 61%, \( P < 0.0001 \) and 20% vs 31%, \( P < 0.05 \), respectively) and higher abortion rates (30% vs 15%, \( P < 0.03 \)) (Strassburger et al., 2009). The difference between Strassburger et al.’s results and those from earlier studies by Palermo and Nagy may lie in the dramatically lower sperm counts in Strassburger’s cryptozoospermic group compared with the oligozoospermic groups in Palermo’s or Nagy’s studies (Palermo et al., 1993; Nagy et al., 1995). It is likely that in the study by Strassburger et al., the very low sperm count made it difficult to identify morphologically normal sperm for injection. Furthermore, men with cryptozoospermia may be more likely to harbor chromosomal abnormalities that may contribute to worse ICSI outcomes (De Braekeleer and Dao, 1991).

An important concern for a subfertile man with abnormal semen parameters is that an underlying genetic component associated with the impaired sperm characteristics may also adversely impact ICSI outcomes. Indeed, a 2008 systematic review and meta-analysis of 2162 cycles of treatment assessing the impact of sperm DNA integrity on results of assisted reproductive treatment demonstrated an adverse effect of sperm DNA damage on the chance of pregnancy, with a diagnostic odds ratio of 1.44 (95% CI 1.03, 2.03) and IVF was not more predictive of the effect than ICSI (Collins et al., 2008). In the same year, a study of 1549 IVF/ICSI cycles concluded that sperm DNA damage was also predictive of pregnancy loss after IVF/ICSI (combined OR 2.48; 95% CI 1.52–4.04; \( P < 0.0001 \)) (Zini et al., 2008). A subset analysis examining only ICSI cycles resulted in the same conclusion (combined OR 2.73; 95% CI 1.43–5.20; \( P < 0.01 \)) (Zini et al., 2008). In 2014, a systematic review and meta-analysis examining 3106 couples found similar results, with high levels of sperm DNA damage being associated with higher miscarriage rates after ICSI (OR 2.68; 95% CI 1.40–5.14; \( P = 0.003 \)) (Zhao et al., 2014). Recently, Oleszczuk et al. (2016) published an update of their series and observed that sperm DNA damage is associated with an increased risk of miscarriage.

**Testicular sperm for ICSI in men with severe oligozoospermia or sperm DNA damage**

In view of the observation of an effect of abnormal sperm DNA integrity on IVF outcomes, Greco et al. commented that ‘no specific treatment for infertility caused by this condition (sperm DNA damage) has yet been proposed’. They hypothesized that the DNA damage in ejaculated sperm begins after spermatozoa are released from Sertoli cells and that sperm recovered directly from the testis would show less damage than ejaculated sperm (Greco et al., 2005). To test this hypothesis, they (i) compared the DNA damage in ejaculated and testicular sperm in two sequential assisted reproduction cycles performed in couples with high levels of ejaculated sperm DNA damage and (ii) evaluated the ICSI outcomes of these couples. Greco et al. reported higher pregnancy rates with ICSI when using testicular rather than ejaculated sperm in couples with high levels of sperm DNA damage. They proposed that the poorer outcome with ejaculated sperm was a result of acquired DNA damage during transit through the epididymis or possibly during ejaculation based on the observed higher frequency of sperm showing detectable DNA damage in ejaculated versus testicular sperm (Greco et al., 2005).

Suganuma et al. conducted experimental studies using a well-characterized animal model with abnormal spermatogenesis (mutant mice with minimal levels of transition nuclear proteins) to investigate the findings of Greco et al. (i.e. that DNA damage in ejaculated sperm begins after spermatozoa are released from the testis). Suganuma et al. (2005) observed that the passage of sperm through the epididymis was associated with a loss of sperm DNA integrity and fertilizing capacity. They speculated ‘that in these infertile animals, the sperm DNA is not fully protected during epididymal passage because the spermatozoa have decreased sperm nuclear compaction due to the higher levels of residual histones and lower levels of disulfide bond formation’. In contrast, in animals with normal spermatogenesis, the passage of sperm through the epididymis was not associated with a similar loss of sperm DNA integrity and fertilizing capacity. As such, Suganuma et al. suggested that in some men (i.e. those with abnormal spermatogenesis), the passage of sperm through the epididymis could be associated with a loss of sperm DNA integrity and fertilizing capacity. Before these papers were published, the post-testicular environment (e.g. the epididymis) was believed to protect sperm and enhance sperm maturation regardless of the quality of spermatogenesis. The observation that administration of selective serotonin reuptake antagonist paroxetine (that affects ejaculation) can adversely affect sperm DNA integrity within weeks supports the mechanism of sperm DNA damage being acquired after testicular sperm production (Tannikut et al., 2010). This new concept (i.e. that the post-testicular environment or epididymal transit can, in selected cases, induce sperm damage) has led clinicians to explore the use of testicular rather than ejaculated sperm for ICSI in men with abnormal spermatogenesis.

Following the report of Greco et al. (2005), additional studies have reported high pregnancy and live birth rates using testicular sperm in men with elevated sperm DNA damage (Esteves et al., 2015; Mehta et al., 2015). Esteves et al. reported the outcomes of their prospective, observational, cohort study of 147 infertile couples. They included couples with mild to moderate idiopathic oligozoospermia (<15 million/ml and >5 million/ml), persistently elevated levels of
sperm DNA damage is associated with poor semen parameters (Zini and Sigman, 2009), several investigators have performed ICSI using testicular sperm in men with necrozoospermia (Negri et al., 2014) and cryptozoospermia (Bendikson et al., 2008; Hauser et al., 2011; Ben-Ami et al., 2013) and have reported high pregnancy and live birth rates in these couples. However, other studies have failed to show that ICSI using testicular sperm is associated with higher pregnancy rates than ICSI using ejaculated sperm in men with cryptozoospermia (Amirjannati et al., 2012; Gnoth et al., 2015). A recent meta-analysis of cryptozoospermic couples has demonstrated that ICSI outcomes with the use of testicular sperm are not better than outcomes using ejaculated sperm in these couples (Abhyankar et al., 2016).

It is unknown whether, globally, it is common practice to perform testicular sperm retrieval (TSR) with ICSI in select groups of non-azoospermic infertility men. In a recent survey of Canadian fertility clinics (unpublished observations presented at the 2015 annual meeting of the Canadian Fertility and Andrology Society), we found that up to 70% of these clinics perform TSR with ICSI in select groups of non-azoospermic men.

**Sperm DNA damage tests to select TSR-ICSI candidates among non-azoospermic couples**

The integrity of sperm DNA is considered to be vital for normal fertilization and embryo development and for successful implantation and pregnancies in both natural and assisted reproduction (Aitken and De Iuliis 2007; Zini and Sigman 2009; Barratt et al., 2010). Tests of sperm DNA damage measure the proportion of cells with DNA damage or fragmentation (i.e. a report of 25% DNA fragmentation means that 25% of the sperm possess DNA damage). It is important to note that the sperm DNA damage in the neat sample (prior to processing) is a better predictor of pregnancy outcome than sperm DNA damage in the prepared or washed sample. This observation suggests that sperm DNA integrity testing reflects the quality of the entire semen sample, not just the few severely damaged sperm detected in the test result. Although a number of studies have suggested utilizing sperm DNA tests in the evaluation of male infertility (Evanson et al., 1999; O’Brien and Zini 2005; Simon et al., 2011), the prognostic value of sperm DNA assessment to predict the results of ART outcomes has not been strong enough to recommend routine testing prior to ART treatment (Practice Committee of the American Society for Reproductive Medicine, 2013).

The current literature on sperm DNA damage and its effect on ART outcomes is still controversial. The meta-analysis by Li et al. (2006) concluded that sperm DNA damage is associated with IVF clinical pregnancy rates but not with ICSI outcomes. Another meta-analysis by Collins et al. (2008) concluded that the sperm DNA damage predicts assisted reproductive outcome but the results of testing would not necessarily affect the decision to proceed with ART. The Practice Committee of the American Society for Reproductive Medicine (ASRM) concluded that the existing data do not support a consistent relationship between abnormal DNA integrity and ART outcomes (Practice Committee of the American Society for Reproductive Medicine, 2013). In contrast, the meta-analysis by Zini et al. provides a clinical indication for evaluating sperm DNA damage prior to fertility treatment. It also provides a rationale for further investigation of the association between sperm DNA damage and pregnancy loss (Zini et al., 2008). A recent meta-analysis by Zhao et al. (2014) also strongly suggests that assays detecting sperm DNA damage should be recommended to those couples experiencing failure to achieve pregnancy.

The lack of agreement on the clinical value of sperm DNA tests is confounded by the heterogeneity of study populations, potential competing factors known to affect pregnancy outcome (e.g. female age) and the diversity of test methods used. Moreover, the lack of standardized test protocols, inter-laboratory variations, the use of wide ranges of threshold values and, to some extent, the limited understanding of what each of the sperm DNA assays actually measure also contributes to the controversy (Zini and Sigman 2009; Barratt et al., 2010; Barratt and De Jonge 2010; Practice Committee of the American Society for Reproductive Medicine, 2013; Zini et al., 2014). Decisions regarding the use of testicular sperm for ICSI should take into account the sperm DNA integrity results and the couple’s overall fertility evaluation, as well as the potential risks of TSR.

**Risks of TESE-ICSI in men with severe oligozoospermia or sperm DNA damage**

Although existing data suggest that there may be some benefit in performing TSR-ICSI in select groups non-azoospermic infertile men, one must remain cognizant of the potential risks involved with TSR techniques (e.g. testicular sperm extraction, TESE, or testicular sperm aspiration, TESA). While testicular fine needle aspiration may appear to be a benign procedure, it has been associated with testicular hemorrhage (Friedler et al., 1997). The complications from conventional TESE vary from transient inflammatory changes or hematomas detected on ultrasound, lasting for up to 6 months post-operatively in up to 80% of patients, to complete devascularization of the testicle (Schlegel and Su, 1997). Other groups performing conventional TESE have reported hypogonadism requiring HRT in 2.5% of patients, testicular atrophy in 25% of patients and chronic testicular changes seen on ultrasound in 23% of patients (Okada et al., 2002). While microdissection TESE has been shown to have fewer complications than conventional TESE, patients undergoing microdissection TESE can still have complications varying from inflammatory and endocrinological changes, lasting up to 12 months, to fibrosis and testicular atrophy (Deruyver et al., 2014).
Unlike ejaculation, which has minimal complications, TSR cannot be considered benign. The use of testicular sperm is typically associated with a lower fertilization rate per injected oocyte than ICSI using epididymal or ejaculated sperm (Oron et al., 2014). For couples with limited ovarian reserve or few retrieved oocytes, the lower fertilization rate can impair the chances of having adequate embryos for transfer. Several investigators have reported that men with oligozoospermia, particularly those with severe oligozoospermia have higher rates of sperm aneuploidy and aberrant sperm DNA methylation (Bernardini et al., 2000; Calogero et al., 2001; Pang et al., 2005; Martin 2006; Faure et al., 2007; Mokanski et al., 2012; Klaver et al., 2013). Moreover, the rate of sperm aneuploidy has been linked to poor reproductive outcomes (recurrent pregnancy loss, aneuploid embryos) (Rubio et al., 2001; Chatziparasidou et al., 2015; Ramasamy et al., 2015). In the context of TSR-ICSI, an important concern is that testicular sperm have a higher rate of aneuploidy than do ejaculated sperm from fertile and infertile men (Bernardini et al., 2000; Rodrigo et al., 2004; Gianaroli et al., 2005; Moskovtsev et al., 2012; Vozdova et al., 2012). As such, it is important to recognize that the use of testicular sperm in couples with OAT (particularly, severe OAT) may increase the genetic risk to embryos and ultimately, the offspring (Platteau et al., 2004). Some studies have suggested offering preimplantation genetic diagnosis in couples with non-obstructive azoospermia and undergoing TSR (Vialard et al., 2012), although existing data have not shown an increase in birth defects for children born from TESE-ICSI, even in non-obstructive azoospermia (Tsai et al., 2011). Clearly, larger studies need to be conducted to better address the genetic and possible epigenetic risk to the offspring associated with TSR-ICSI.

Importance of evaluating and treating causes of severe male factor infertility

A number of studies have shown that a short abstinence regimen may lower sperm DNA damage in infertile men, including men with high levels of sperm DNA damage. Gosalvez et al. (2011) observed that when men submitted a semen sample following a short abstinence period (24 and 3 h) they had a lower level of sperm DNA damage than after a standard or recommended abstinence time (4 days). Pons et al. (2013) evaluated 35 men with high levels of sperm DNA damage (>30% DNA fragmentation) and reported that DNA fragmentation decreased to normal values in one of the three subsequent semen samples submitted following a 1-day abstinence period. Uppangala et al. (2016) also observed that a shorter abstinence period is associated with a lower level of sperm DNA fragmentation in fertile men. However, these investigators noted that spermatozoa collected after a short incubation period had poorer chromatin compaction and a greater susceptibility to DNA damage from prolonged in vitro incubation (Uppangala et al., 2016). Taken together, these observations suggest that spermatozoa collected following a short abstinence period are relatively immature yet possess relatively low levels of sperm DNA damage (compared with spermatozoa collected following a standard abstinence period). These observations also suggest that a longer abstinence period with prolonged epididymal storage may induce DNA damage.

Clinical evaluation of the infertile male can identify a correctable cause of infertility and allow subsequent treatment of these men to improve sperm parameters (including sperm DNA damage) and potentially avoid TSR with ICSI. Men with abnormal sperm DNA damage and infections will gain substantial benefits from treatment of the inflammatory condition (Moskovtsev et al., 2009). One of the common correctable factors in infertile men is clinical varicocele. By use of systematic review and meta-analysis, several investigators have demonstrated that repair of clinical varicocele is associated with improved semen parameters and natural pregnancy rates (Bazzeem et al., 2011; Kroese et al., 2012). Moreover, a systematic review of varicocelectomy studies has shown that correction of clinical varicocele in men with infertility is also associated with a significant decrease in sperm DNA damage (Zini and Dohle, 2011). Unfortunately, many men with abnormal sperm DNA integrity and clinical varicoceles who undergo varicocelectomy will still have substantial sperm DNA damage after repair. Other than treating correctable factors, infertile men may be offered oral antioxidant supplements. Oral antioxidant therapy can lower the levels of sperm DNA damage in infertile men (Zini et al., 2009; Talevi et al., 2013; Gual-Frau et al., 2015). A recent meta-analysis has shown that oral antioxidant therapy may improve semen parameters and natural pregnancy rates, although the strength of the evidence is poor (Showell et al., 2014).

How do we select and counsel potential non-azoospermic candidates for TSR?

In the management of couples who undergo ART treatment, prior failed cycles are often managed by changing the cycle, with a different sperm source, altered ovarian stimulation or other variations in the IVF process. The evidence basis for these interventions is poor. A recent review of couples with very poor sperm parameters suggested no benefit from TSR as an intervention for men with cryptozoospermia (Abhyankar et al., 2016). The data supporting use of TSR for couples with very poor sperm parameters and prior ICSI failures are weak in the absence of sperm DNA damage. Given the lower fertilization rate with testicular sperm, the lack of a strong rationale for using testicular sperm in the absence of DNA damage and limited outcomes data, TSR should be used with much caution despite prior IVF failures.

Even in the setting of abnormal sperm DNA fragmentation and prior failed IVF cycles, the management of couples can be very controversial. Treatable male factor conditions should be identified and addressed, where feasible. Since sperm DNA integrity is better with testicular sperm, TSR may be considered in couples with significantly abnormal sperm DNA integrity and prior failed IVF cycles. It should be remembered that the data supporting TSR are based on a relatively small number of fair quality studies indicating that (i) couples with very poor sperm parameters and/or sperm DNA damage have poor ICSI pregnancy rates and higher rates of ICSI pregnancy loss and (ii) these same couples, in some cases, experience pregnancy after ICSI with the use of testicular sperm. Based on the fair quality of the available studies, we believe that clinicians should be thoughtful with their plans to proceed to TSR-ICSI.

To date, there are no established criteria to guide clinical decision-making in this context. Specifically, there are no clear thresholds for
sperm concentration, motility or DNA integrity below which TSR-ICSI is deemed beneficial. Therefore, clinicians can only rely on their judgment when deciding to proceed to TSR-ICSI as opposed to ICSI with ejaculated sperm. Clinicians should recognize the variability of sperm DNA damage test results (different assays and variable assay thresholds) and be very careful in interpreting sperm DNA test results to identify TSR candidates. It is also critical that a reliable sperm DNA assay with a validated threshold is used. Every effort to reduce sperm DNA damage should be undertaken before performing TSR. Clinicians will need to consider the potential risks of a testicular biopsy or testicular aspiration (e.g., bleeding, infection, pain, hypogonadism) and the unknown genetic or epigenetic risks of using testicular rather than ejaculated sperm. Ultimately, it is important that patients be counseled that the use of TSR is a potentially beneficial treatment approach but it remains experimental in the absence of substantial sperm DNA fragmentation.

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Dr A.Z. is a shareholder in YAD Tech (a Canadian Nutraceuticals Company). Dr P.N.S. is a Medical Advisory Board member for Theralogix, Inc. (a U.S. Nutraceutical manufacturer). Dr A.H.A.-M. and Dr P.V.B. have no conflicts of interest.

References


Zini A, Boman JM, Belzile E, Ciampi A. Sperm DNA damage is associated with an increased risk of pregnancy loss after IVF and ICSI: systematic review and meta-analysis. *Hum Reprod* 2008; **23**:2663–2668.

