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COMMENTARY

Male Infertility

When to pull the trigger in nonazoospermic infertile men undergoing intracytoplasmic sperm injection?

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Before the era of intracytoplasmic sperm injection (ICSI), few options were available for men with severe infertility to father a biological child. Introduced in 1992 as a modification of conventional *in vitro* fertilization, ICSI has enabled men with low sperm quantity and quality to have their own biological offspring.[1] Among nonazoospermic infertile men, ICSI has been traditionally carried out with ejaculated sperm. By contrast, methods to harvest sperm from the epididymides and testes are used for ICSI in men with azoospermia-related infertility.[2]

However, as our experience has accumulated, reports of an association between semen quality and ICSI outcomes have increased steadily.[3,4] Concerns of a possible role of the paternal gamete on ICSI outcomes have led many authors to investigate the utility of testicular sperm retrieval in nonazospermic men with defective sperm quantity or quality.[5,6] The evidence to date indicates that among infertile men, sperm chromatin integrity progressively decreases as sperm transit across the genital tract.[6] The mechanisms are not fully understood, but oxidative attack in the epididymis, after sperm release from the seminiferous tubules, is a major source of DNA damage, increasing the frequency of DNA-damaged sperm as well as the amount of damage per sperm in ejaculated sperm compared with testicular sperm.[7]

Within this issue of *Asian Journal of Andrology*, Alharbi *et al.*[8] retrospectively reviewed their single-institution database concerning 187 couples with ICSI failure who underwent sperm injection with either ejaculated ($n = 135$) or testicular sperm ($n = 52$) on subsequent cycles. Testicular sperm aspiration carried out under local anesthesia was overall successful in retrieving viable sperm for injection, with no apparent complications. The indication for sperm retrieval was sperm DNA fragmentation (SDF) greater than 15%, as measured by the sperm chromatin structure assay (SCSA) on neat semen. The authors compared ICSI pregnancy outcomes between the testicular sperm group to those of ejaculated sperm with known ($n = 48$) and unknown ($n = 87$) SDF data and concluded that no significant difference was noted in live birth rates (LBR) among the groups.

Data of Alharbi *et al.*[8] contrast with the results of other studies on the matter concerned, all of which suggest that ICSI with testicular sperm is superior to ICSI with ejaculated sperm to overcome infertility in men with high SDF (reviewed by Lopes and Esteves[9]). This observation has led us to examine their paper more carefully in an attempt to find possible reasons for such discrepancy.

First, Alharbi *et al.*[8] used the SDF cutoff point of 15% to include patients with the so-called "high SDF," whereas studies using similar assays utilized a cutoff of 30%. The authors justified the 15% cutoff based on two studies that used SDF thresholds of 15%. The first study showed that couples with SDF values >15% had either a longer time to natural pregnancy - or did not achieve natural pregnancy at all - than couples with values <15%. Thus, the SDF thresholds of 15% related to natural pregnancies rather than ICSI pregnancies.[10] Indeed, Evenson,[11] who developed the SCSA assay and was the leading author of the above study, has demonstrated that the odds for pregnancy by assisted reproductive technology (ART) decrease as SDF values increase, particularly in patients with values of 30% and higher. The second study[8] applied a different SDF assay (terminal deoxynucleotidyl transferase [TDT]-mediated dUTP-biotin nick end-labeling [TUNEL] assay);[12] TUNEL thresholds have been suggested to be lower than those of SCSA and sperm dispersion test (SCD). The Comet assay has a similar threshold to SCSA (27%), but this is based on the actual damage per sperm, not the proportion of damaged sperm per sample. In fact, it has been suggested that this novel way of assessing sperm DNA damage might be the ideal method to identify men at risk of poor reproductive outcomes in ICSI.[13] Thus, the inclusion of approximately 30% of men with arguably high SDF values (SDF values between 15% and 30%) in the study by Alharbi *et al.*[8] might have diluted the positive effect of ICSI using testicular sperm (Testi-ICSI).

The above observations are consistent with the examination of Alharbi's results concerning a subgroup analysis of patients with SDF values >30%.[8] Thirty-three men fit these criteria and had ICSI outcomes compared between Testi-ICSI ($n = 25$) and ICSI using ejaculated sperm (Ejac-ICSI, $n = 8$). The clinical pregnancy rate was remarkably higher after ICSI with testicular sperm than ejaculated sperm (48% *vs* 25%) even though statistical significance was not achieved, which was due to a small sample size resulting in an imprecise estimate of treatment effect. Indeed, we conducted a *post hoc* analysis using the authors' data, which showed that the ability of their trial to detect a difference between the two groups was only 18.6%. Using the authors' reported pregnancy rates, a minimum of 68 patients per group would be needed to have sufficient statistical power to detect a treatment effect.

Second, the mean SDF values in the group of men subjected to Testi-ICSI was significantly higher than that in the Ejac-ICSI group (37.6% *vs* 26%; $P < 0.001$). Thus, sperm quality concerning DNA damage was worse in the former, which might have biased results in favor of the ejaculated sperm group. Indeed, Evenson[11] has shown that miscarriage rates in ART cycles are significantly higher if SDF values are close to 40% or above. Moreover, we question if female age was a hidden confounder factor. In Alharbi's study, maternal age was, on average, below 35 years. However, couples usually undertake ICSI with testicular sperm when the women are older. If these women were classified <38 years and 38 years and over, the outcomes might have been quite different because the oocyte apparatus to repair sperm DNA damage is less efficient as both ovarian reserve and maternal age increase.[14] Thus, the use of testicular sperm for ICSI may be of particular significance for the latter.

We are somewhat surprised that, in light of these observations, Alharbi *et al.*[8] concluded that Testi-ICSI provided no significant advantage over Ejac-ICSI in men with high SDF. We, therefore, propose an alternative interpretation of the data, stating that the study of Alharbi strongly suggests that pregnancy outcomes are affected by the type of sperm used for ICSI in men with SDF values >30%. The use of testicular sperm for ICSI on these couples increased clinical pregnancy rates and live birth rates. Moreover, in the authors' overall population of men with SDF values >15%, whose sperm quality was remarkably poorer in

the group of men subjected to ICSI with testicular sperm (*vs* ejaculated sperm), Testi-ICSI was able to provide comparable clinical pregnancy rates and live birth rates. We would suggest that individualization of the type of sperm used for ICSI is superior to "a one-size-fits all" in nonazoospermic men with poor sperm DNA integrity.

COMPETING INTERESTS

SCE serves the editorial board of *Asian Journal of Andrology* since 2015. The author declares no competing interests. SEML is an employee of Examenlab Ltd., a university spin-out company with a commercial interest in sperm DNA damage.

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