Editorial

Management of patients with high sperm DNA damage

The interest in the use of sperm DNA integrity as a predictor of fertility potential is on the rise. Clear differences in the levels of sperm DNA damage have been observed between fertile and infertile men. Men with a high percentage of DNA fragmentation have very low potential for in vitro and in vivo fertility. Moreover, DNA fragmentation is linked to effects on embryonic development, implantation and risk of recurrent miscarriages.

The potential causes of sperm DNA damage are complex with multiple factors acting at both the intra- and post-testicular levels. Oxidative stress, defective sperm chromatin packaging and disordered abortive apoptosis are the three putative mechanisms most commonly associated with DNA damage.

Human spermatozoa are susceptible to oxidative stress because of a high content of polyunsaturated fatty acids in their plasma membranes. Damaging effect of reactive oxygen species (ROS) on sperm DNA have been reported. Antioxidants present in seminal plasma may control ROS generation to some degree, but the production of excessive amounts of ROS may overcome the antioxidant protective activities of seminal plasma leading to oxidative stress. Many antioxidants play an important role in various biological processes; enzyme production, DNA synthesis, testicular development and sperm maturation are microelement and vitamin dependent. Antioxidants added as dietary supplements have been shown to reduce the degree of oxidative damage by improving cellular redox equilibrium and removing free radicals. The literature on the benefit of antioxidant therapy in male infertility is inconclusive; both positive effects and no significant effect having been reported.

Several studies reported a positive effect of antioxidants, for example, a decrease in DNA damage in 76 per cent of patients with moderately increased DNA fragmentation (> 15%) after oral treatment with vitamins C and E have been observed. In contrast, others could not confirm these findings. The variations in response to the treatment may be related to individual differences in nutrient absorption and metabolism of the antioxidants, as well as to failure of the antioxidant system or enzyme production in some patients. Multiple antioxidants acting through different mechanisms on diverse free radicals have been suggested as a therapeutic approach in the treatment of male infertility.

Since the introduction of intracytoplasmic sperm injection (ICSI), many traditional diagnostic algorithms have been underutilized because of their perceived poor predictive value in achieving fertilization. However, there has been renewed interest in the use of tests beyond semen analysis due in part to the increase in the number of studies indicating the risk of anomalies in children born after ICSI. One of the possible explanations in the increase of health problems in children born after ICSI is the negative effect of the DNA damage, because ICSI forgoes spermatozoon selection and bypasses normal sperm-egg interactions allowing even spermatozoa with low quality to initiate a successful pregnancy. In patients with high sperm DNA damage this problem may arrive more often or be more severe.

The challenge in the management of patients with elevated DNA damage increases proportionately with the rise of such damage. Several methods designed to separate superior spermatozoa have been recommended, based on the heterogeneous characteristics of single ejaculate in terms of the semen and DNA quality. Some methods of sperm separation are routinely used in the preparation of ejaculate as a part of sperm preparation for the assisted reproductive techniques (ART). Both swim-up and density gradient centrifugation methods have been evaluated for DNA damage. For example, swim-up sperm preparation was shown to better eliminate DNA defective spermatozoa. DNA damage...
dropped from 12 to 5.5 per cent after swim-up\textsuperscript{12}. In another study, DNA fragmentation was 28 per cent in neat semen and fell to 24 per cent after gradient density preparation\textsuperscript{13}.

Several novel techniques to choose the best spermatozoa from ejaculate have been introduced. One of these methods is the separation of spermatozoa by magnetic-activated cell sorting (MACS). The technique is based on the ability of spermatozoa to express the apoptotic marker phosphatidylserine, which binds to Annexin-V-conjugated micro-beads. Spermatozoa with signs of apoptosis (DNA fragmentation) could be separated by a magnetic field to Annexin-V positive and negative fractions and could be used in ART procedures\textsuperscript{14}.

Another innovative method which has been introduced is the electrophoretic separation of spermatozoa. The method is based on the observation that mature spermatozoa are more electronegative due to the negatively charged glycocalyx rich in sialic acid residues. Spermatozoa are separated by traveling toward the positively charged cathode and away from the negatively charged anode. It was shown that electrophoretically isolated spermatozoa have low DNA damage revealed by TUNEL assay. First pregnancy and normal birth were reported after ICSI using electrophoretically isolated spermatozoa\textsuperscript{15}.

Another promising method of selecting spermatozoa was developed specifically for ICSI procedures. The “High-magnification ICSI” method of selecting spermatozoa employs a high-magnification digitally enhanced optical system allowing ×6600 magnification which can reveal intranuclear vacuoles possibly associated with alterations in chromatin packaging. ICSI performed with this system has been shown to significantly increase pregnancy rates compared with conventional in vitro fertilization (IVF) technique in patients with high DNA fragmentation\textsuperscript{16}.

Retrieval of testicular spermatozoa, which generally have lower levels of DNA damage, has been also suggested for patients with markedly increased DNA damage. The reproductive outcome of two subsequent ICSI attempts using ejaculated and testicular spermatozoa in 18 patients were compared. The pregnancy rate using testicular spermatozoa was 44 versus 6 per cent using ejaculated spermatozoa, and the implantation rate was 22 versus 2 per cent or one pregnancy which spontaneously aborted\textsuperscript{17}. However, these findings have to be confirmed in larger populations of patients, as no differences in the outcomes of ICSI with epididymal, testicular and neat spermatozoa were reported\textsuperscript{18}. Positive progress has been recently made in the management of patients with high sperm DNA damage. At this time however, it is still impossible to predict the outcome of this treatment to the particular sample or individual, regardless of the method of treatment designed to minimize the sperm DNA damage. Moreover the chance of passing unbalanced or damaged paternal DNA, which might affect the normal embryo development, implantation and even health of the offspring with use of any of these described method could be quite moderate. Development of specific screening tests for monitoring and minimizing the potential risk of such DNA damage is essential.

Further research is necessary to obtain deeper knowledge about the mechanisms and effects of sperm DNA damage and to satisfy demands for evidence-based guidelines for the management of patients with elevated DNA damage.

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\textbf{References}


