



Significance Of Human Sperm Chromatin Damage In Assisted Reproduction



Ashok Agarwal and Shyam S.R. Allamaneni

*Center for Advanced Research in Human Reproduction, Infertility and Sexual Function,
Glickman Urological Institute and Department of Obstetrics and Gynecology,
Cleveland Clinic Foundation, Cleveland, Ohio, USA
e-mail: agarwaa@ccf.org*

The quality of sperm DNA is very important in maintaining the reproductive potential of men. Sperm DNA is known to contribute one half of the genomic material to offspring. Normal sperm genetic material is required for fertilization, embryo and fetal development and postnatal child well being. Abnormal DNA can lead to derangements in any of these processes. Sperm DNA is resistant to many types of insults that occur during its journey from the testis to the time it reaches the oocyte for fertilization. Recent reports have raised concern about decreasing male fertility caused by genomic abnormalities. There are reports of increased congenital anomalies and testicular cancer in children (Aitken and Sawyer, 2003). The defects in the genomic material may take the form of condensation or nuclear maturity defects, DNA breaks or DNA integrity defects and sperm chromosomal aneuploidy (Perreault et al, 2003).

Recently, sperm DNA has been recognized as an independent measure of sperm quality that may have better diagnostic and prognostic capabilities than standard sperm parameters for both in vivo and in vitro fertility. The cause of infertility in infertile men with normal semen parameters could be related to abnormal sperm DNA (Alvarez, 2003).

Therefore, the evaluation of sperm DNA integrity, in addition to routine sperm parameters, could add further information on the quality of spermatozoa. The damage to sperm DNA is critical in the context of assisted reproductive techniques (ART), which are increasingly used to treat infertile couples (Sakkas et al, 2003). The main disadvantage of ART is that they bypass the natural selection barrier that is present throughout female reproductive tract until sperm enter the oocyte (Chandley and Hargreave, 1996). Nature has created multiple obstacles that allow only the fittest sperm to reach and fertilize an oocyte. With ART, sperm with abnormal genomic material can reach the genetic material of the oocyte with minimal effort (in vitro fertilization; IVF) or no effort (intra cytoplasmic sperm injection; ICSI). Genetically damaged spermatozoa may be able to fertilize an oocyte when they are directly injected into it (Twigg et al, 1998).

Mechanisms of sperm DNA damage

Normally, sperm DNA is very stable in the nucleus because of its characteristic organization (Agarwal and Said, 2003). The cause of DNA damage in sperm may be due to disease, drug use, high fever, elevated testicular temperature, air pollution, cigarette smoking and advanced age.

Various hypotheses are being proposed as molecular mechanisms of sperm DNA damage. The most important mechanisms of sperm DNA damage are abnormal chromatin packaging, reactive oxygen species (ROS) and apoptosis (Sakkas et al, 1999; Shen and Ong, 2000). Reactive oxygen species in particular have received special attention due to their significant role in both the physiology and pathology of human reproduction (Agarwal et al, 2003). Oxidative stress occurs when there is an excessive production of ROS by leukocytes or abnormal spermatozoa and/or decreased antioxidant capacity of semen. Many studies have reported that ROS are a major cause of sperm DNA damage (Barroso et al, 2000; Agarwal and Said, 2003; Moustafa et al, 2004).

Evaluation of DNA damage

Currently, there is extensive research on quantifying the amount of abnormal DNA present in human spermatozoa. Multiple techniques are reported to measure sperm DNA defects in human spermatozoa (Evenson et al, 2002; Perreault et al, 2003). Some methods like terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-nick end labeling (TUNEL), comet, in-situ nick translation, and sperm chromatin structure assay (SCSA) can evaluate the integrity of sperm DNA.

Others techniques, like staining with aniline blue, toluidine blue, and chromomycin A3, help identify the packaging defects of sperm chromatin.

ART & DNA damage

Sperm DNA damage has attracted a great deal of attention due to recent reports correlating the degree of DNA damage with various fertility indices such as the fertilization rate, embryo cleavage rate, implantation rate, pregnancy rate and live birth rate of the offspring. If sperm DNA is unable to decondense after entering the ooplasm, fertilization may fail or a post-fertilization failure could occur due to defective sperm DNA (eg, poor embryo quality) (Tomsu et al, 2002). Pregnancy loss may occur with increase in degree of sperm DNA damage and this could be the cause of unexplained pregnancy loss in some patients (Carrell et al, 2003). Pregnancy and live births after IVF/ICSI are also associated with the degree of spermatozoal DNA damage (Tomlinson et al, 2001). In addition, the degree of DNA damage can affect the ability of a couple to conceive naturally (Evenson et al, 1999; Spano et al, 2000).

Intrauterine insemination. The degree of sperm DNA fragmentation and stability, which were determined using TUNEL and acridine orange staining, was used in predicting the success of intrauterine insemination (IUI) outcome (Duran et al, 2002). Authors reported that the degree of DNA fragmentation after sperm preparation was significantly lower in the samples that initiated a pregnancy than in those that did not. No woman became pregnant when samples with >12% of sperm with fragmented DNA were used for insemination.

IVF. A number of studies have shown that the sperm DNA damage affects the fertilization and pregnancy after the IVF. Sun et al (1997) evaluated sperm DNA fragmentation using the TUNEL assay and found that up to 40% of sperm from semen samples obtained in an infertility clinic

contained fragmented DNA. They found a negative relationship between the percentage of sperm with DNA fragmentation and the fertilization and embryo cleavage rate in couples undergoing IVF.

Defects in sperm condensation may lead to defective decondensation of sperm DNA in ooplasm and impairment of fertility (Haidl and Schill, 1994). The success rate of IVF could be predicted using the DNA damage levels in spermatozoa (Esterhuizen et al, 2000). Henkel et al reported that even though sperm DNA fragmentation did not correlate with the fertilization and embryo fragmentation rates, the pregnancy rates in IVF patients were significantly low when TUNEL-positive spermatozoa (>36.5% TUNEL-positive spermatozoa) were used (Henkel et al, 2003). These studies support Twigg et al's finding that spermatozoa with damaged DNA can fertilize an oocyte and form the pronucleus after IVF and ICSI (Twigg et al, 1998). However, depending on the degree of DNA damage, embryo development is affected in later stages and may lead to embryo death in severe cases.

ICSI. DNA damage detected by TUNEL is negatively correlated with the ICSI fertilization rate (Lopes et al, 1998). A fertilization rate of less than 20% was seen in patients with high sperm DNA damage (> 25%). The proportion of sperm with DNA fragmentation influences the fertilization rate and the implantation rate of ICSI-derived embryos at a threshold value above 10%. No pregnancies were initiated if >20% of selected sperm were TUNEL positive, suggesting that DNA damage may have a good predictive value in cases of successive implantation failures involving good quality embryos (Benchaib et al, 2003).

The DNA fragmentation index (DFI) was negatively correlated with fertilization ($r = -0.70$; $P = 0.03$) and embryo quality ($r = -0.70$; $P = 0.03$) after IVF and ICSI. The percentage

DFI was lower in the infertile men who initiated a clinical pregnancy after assisted conception [21 (13, 25)] than in those who did not initiate a pregnancy [38 (28, 43); $P = 0.001$] (Saleh et al, 2003).

SCSA is predictive of negative pregnancy outcome when the spermatozoa in the neat semen showed acid-induced DNA denaturation of $\geq 27\%$ DFI (Larson-Cook et al, 2003). However, some studies have failed to show the negative effect of damaged sperm DNA on fertilization and pregnancy outcome (Angelopoulos et al, 1998; Host et al., 2000; Henkel et al, 2003). Hammadeh et al found no difference in the fertilization, cleavage and pregnancy rates in patients undergoing ICSI between patients with different degrees of sperm nuclear condensation (Hammadeh et al, 1996; Hammadeh et al, 2001).

Clinical significance of sperm DNA damage

With the advent of ART procedures that require few spermatozoa, traditional semen parameters like count have become less important in the evaluation of sperm quality. The ART procedures bypass natural selection mechanisms, which increases the chance of sperm with abnormal genomic material fertilizing an oocyte (Aitken, 1999). In view of the changed circumstances, alternate methods which can evaluate sperm quality such as assessment of sperm DNA damage has assumed greater significance. Extensive data now exists on the relationship between sperm DNA damage and outcomes after ARTs. Most of the studies have shown a significant negative association with fertility outcome indices after ARTs.

Some of the ICSI studies have found a lack of correlation between fertilization rates and DNA damage, suggesting that ICSI bypasses the natural selection mechanisms and allows spermatozoa with DNA damage to fertilize oocytes. However most of these same studies have

reported an association between pregnancy rates and sperm DNA damage indicating that the embryo employs certain mechanisms to prevent defective genomic material from passing on to the offspring. The disparity in observations may also be explained by the heterogeneity of the technique used to measure DNA damage, and use of ART patients with different etiologies of infertility.

When spermatozoa with extensive DNA damage are used, the embryo may fail to develop or implant in the

uterus or it may be aborted naturally at a later stage. Even when sperm with minimal DNA damage are used, fetal development can be affected at later stages, resulting in a child with congenital anomalies.

Summary

Evidence now exists in the literature to show that sperm DNA damage influences the fertility outcome after ART procedures. Even if spermatozoa with abnormal DNA fertilize an oocyte and live birth occurs, there is a

possibility of congenital abnormalities in the offspring. Therefore, it is prudent to check for DNA damage in infertile patients undergoing ARTs. The protocols used to detect chromosomal abnormalities and DNA damage should be standardized to allow their routine use in clinical laboratories (Perreault et al, 2003). The results of the sperm DNA damage evaluation may help the physicians better counsel infertile couples referred for assisted conception about their chances of having a live birth of a healthy baby. □

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