

The effect of sperm DNA damage on assisted reproduction outcomes

A review

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The quality of sperm DNA is very important in maintaining the reproductive potential of men. Sperm DNA is resistant to many types of insults that occur during its journey from the testis to time it reaches the oocyte for fertilization. During natural conception, a selection process occurs that allows only sperm with intact DNA to fertilize an oocyte. When assisted reproductive techniques (ART) are used, some or all of this selection process is bypassed. As a result, sperm with damaged DNA may fertilize an oocyte. Damage to sperm nuclear DNA negatively affects assisted and natural fertility. Increasingly, sperm DNA is being recognized as an independent measure of sperm quality that may have better diagnostic and prognostic capabilities than standard sperm parameters. This review summarizes the available evidence for the role of sperm DNA damage in assisted fertility. Two important facts are obvious from the available evidence: 1) men with spermatozoal DNA damage appears to have a decreased ability to father offspring, 2) spermatozoa with abnormal DNA can fertilize an oocyte, which may progress to a live birth. Infertile men who are planning to undergo ART procedures with their partner should have their sperm evaluated for DNA damage. The results of this evaluation may be used to counsel the couple about their chances for live birth and for genomic abnormalities in their offspring.

Key words: DNA damage - Spermatozoa, infertility, male - Reproductive techniques, assisted - Sperm injections, intracytoplasmic - Fertilization *in vitro* - Pregnancy.

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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.¹ Sperm DNA is known to contribute one half of the genomic material to offspring. Thus, 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. The abnormality or defect in the genomic material may take the form of condensation or nuclear maturity defects, DNA breaks or DNA integrity defects and sperm chromosomal aneuploidy.² Routine semen parameters may not always reflect the quality of sperm DNA. Men with normal spermograms may still be infertile; the cause could be related to abnormal sperm DNA.³ Sperm DNA integrity may be evaluated in addition to routine sperm parameters to indicate the quality of spermatozoa.

The importance of sperm DNA has become more obvious in the context of assisted reproductive techniques (ART), which are

used by infertile couples.⁴ The main disadvantage of ART is that they bypass the natural selection barrier that is present throughout the male and female reproductive tracts and until sperm enter the oocyte.⁵ 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.⁶ When spermatozoa with extensive DNA damage are used, the embryo may fail to develop or implant in the uterus or it may be naturally aborted 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 abnormalities.³ Some studies have reported that babies born with the help of ICSI have a higher risk of chromosomal abnormalities and major and minor birth defects.^{7,8} In view of its importance, spermatozoa DNA damage should be evaluated along with routine semen parameters, which may help counsel infertile couples referred for assisted conception about their chances of success (*i.e.*, the live birth of a healthy baby).^{4,9}

DNA damage and reactive oxygen species

Sperm DNA is organized in a unique pattern that keeps the chromatin in the nucleus compact and stable.¹⁰ Various mechanisms can damage sperm DNA. Abnormal chromatin packing, reactive oxygen species (ROS) and apoptosis are the most discussed causes of DNA damage.^{10,11} Reactive oxygen species, in particular, have received special attention due to their significant role in both the physiology and pathology of human reproduction.¹² Spermatozoa are particularly susceptible to ROS-induced damage because their plasma membranes contain large quantities of polyunsaturated fatty acids and their cytoplasm contains low concentrations of

scavenging enzymes.¹³ In addition, deoxyribonucleic acid bases and phosphodiester backbones are susceptible to peroxidation,¹⁴ but the characteristic tight packaging of the sperm DNA may offer some protection against oxidative stress.¹⁵ Oxidative stress occurs when production of ROS by leukocytes or spermatozoa is excessive and/or the antioxidant capacity of semen decreases. Many studies have reported that ROS are a major cause of sperm DNA damage.¹⁶⁻²⁰

Measurement of DNA damage

For many years, researchers have been studying how to best quantify the amount of abnormal DNA that is present in human spermatozoa and relate the results to fertility. Several techniques can be used to study DNA defects in human spermatozoa.^{2,21} Staining with aniline blue, toluidine blue, and chromomycin A3 can identify sperm chromatin packaging defects. The integrity of sperm DNA can be evaluated with the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-nick end labeling (TUNEL), comet, *in-situ* nick translation, or sperm chromatin structure assay (SCSA) techniques. In the TUNEL technique, terminal deoxynucleotidyl transferase (TdT) incorporates dUTP biotinylated deoxyuridine to 3'-OH at single- and double-strand DNA breaks to create a signal, which increases with the number of DNA breaks. With the comet assay, DNA damage is quantified by measuring the displacement between the genetic material of the nucleus comet head and the resulting tail. In the SCSA technique, the extent of DNA denaturation following heat or acid treatment is determined by measuring the metachromatic shift in fluorescence after acridine orange staining. Sperm aneuploidies are usually detected using the fluorescence *in situ* hybridization (FISH) technique.

DNA damage and fertility

The importance of sperm DNA damage was brought to light when studies correlated

the degree of DNA damage with various indices of fertility 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 not take place or a postfertilization failure may occur when sperm DNA is defective (*e.g.*, defective embryo cleavage and development). Pregnancy loss may occur with increase in degree of sperm DNA damage and may be the cause of unexplained pregnancy loss in some patients.²² Pregnancy and live births after IVF/ICSI are also associated with the degree of spermatozoal DNA damage. In addition, the degree of DNA damage can also affect the ability of a couple to conceive naturally.^{23, 24}

Published reviews discussing sperm DNA damage and ART are narrow in their scope because the authors have quoted only a limited number of studies. In addition, the information is part of a broader subject, sperm DNA damage in male infertility, which includes mechanisms and measurement of DNA damage and its relationship with standard semen parameters. Thus, we attempted to focus this review on summarizing the role of DNA damage in the context of ART. In this review, we have attempted to gather and present available published literature on the role of sperm DNA damage in ART.

A meticulous search of the literature was conducted searching the MEDLINE database using PubMed with different key words (DNA, ART, IVF, ICSI, IUI [intra uterine insemination], pregnancy, fertilization, etc.). We reviewed the references cited in the studies identified by a PubMed search. We initially identified a large number of articles, which we short-listed to 590. After reviewing the abstract of each article, we narrowed them to 170 articles. We carefully read the full texts of these articles. Finally, we identified 46 studies in the English language literature that were related to human sperm DNA damage and fertility outcomes after ART procedures.

We initially divided the published studies from the literature based on the type of ARTs that were used because this approach was

considered useful for clinicians. The studies were then further divided based on whether they had reported the influence of DNA damage on any assisted reproduction parameter or showed a lack of effect. We included a studies with effect category when one of the assisted reproduction parameters was found to be affected by DNA damage. Studies were sub-divided based on the type of DNA defect being detected (condensation or nuclear maturity defects/DNA breaks or DNA integrity defects/sperm chromosomal aneuploidy). A variety of techniques were used in the studies to determine DNA damage in spermatozoa. The results of our review are reported below.

DNA damage and IUI

Duran *et al.* studied the degree of sperm DNA fragmentation and stability, which were determined using TUNEL and acridine orange staining, in predicting the success of IUI outcome.²⁵ They 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.

Levels of DNA fragmentation index (DFI) were found to be negatively correlated with the overall pregnancy rate in the patients undergoing IUI, IVF and ICSI ($p < 0.0001$).²⁶ In a recent study, DNA damage as measured by SCSA was found to predict the outcome of IUI. The chances of achieving pregnancy were significantly lower when patients had spermatozoa with a DFI of >27% and HDS (high DNA stainable) >10% compared with patients having a DFI $\leq 27\%$ and HDS $\leq 10\%$.²⁷

DNA damage and IVF

STUDIES SHOWING EFFECT

Correlation with DNA condensation defects.—Histone proteins are replaced by protamines when sperm DNA condenses. This enables the DNA to properly decondense after entering into oocyte and participate in the fertilization process. Using the aniline

blue staining technique, histones can be detected in spermatozoa with DNA that has improperly condensed. When Haidl *et al.* used this technique, they found a high degree of correlation ($r=0.826$) between the extent of normal chromatin condensation and the fertilization rate.²⁸ Moreover, normal condensation was seen in all men with a 100% fertilization rate, which emphasizes the importance of this process. In another study that used the aniline blue staining technique, condensation was a good predictor of the fertilization potential of sperm, cleavage rate and pregnancy rate following IVF.²⁹

Liu *et al.* found a good correlation between the percentage of sperm with normal DNA and IVF rates ($r=0.302$, $p<0.001$). They also noted that sperm bound to zona pellucida had low amounts of DNA damage. It is possible that the human zona pellucida selectively binds sperm with a mature nuclei and normal DNA.³⁰

Filatov *et al.* reported a relationship between the fertilizing potential of spermatozoa and defects in chromatin packing, which were detected using ethidium bromide stain. The quality of sperm chromatin packing did not influence the fertilization rate and percentage of cleaved embryos, but it did affect pregnancy rates after IVF.³¹ This suggests that some mechanism may prevent an embryo from developing when the organization of genetic material in male germinal cells is abnormal.

Esterhuizen *et al.*³² reported a significant negative correlation between the degree of defective chromatin packaging (chromomycin A³ staining) and fertilization rates after IVF ($r=-0.51$, $p=0.0001$). The success rate of IVF could best be predicted when the chromomycin A³ staining value was of greater than 50%. At this cutoff value, the area under curve to differentiate fertilization of less than or more than 50% of metaphase II oocytes was 90%.³²

Studies involving non-fertilized oocytes provided valuable information on the causes of fertilization failure. Esterhuizen *et al.*³³ examined 170 non-fertilized oocytes from couples attending an IVF program. The authors noted that, in the patients with a high

degree of defective chromatin packaging, the chances of finding sperm in the ooplasm decreased and the chances of sperm DNA decondensation failure increased.³³ The degree of sperm chromatin package defects was determined using chromomycin A³ fluorescence.

Correlation with DNA structural integrity and breakage.—Sun *et al.* 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 association between the percentage of sperm with DNA fragmentation and the fertilization and embryo cleavage rate in couples undergoing IVF.³⁴

Duran *et al.* calculated an acridine orange score based on the susceptibility of DNA to undergo denaturation after incubation in tamponade solution. The acridine orange score was significantly and negatively correlated with fertilization rates after IVF ($r=-0.5831$, $p<0.001$).³⁵ Another study showed that single-stranded DNA (ssDNA) detected by a low incidence (<50%) of green acridine orange fluorescence negatively affected the fertilization process in a classic IVF program.³⁶

Tomlinson *et al.* found that the mean percentage of DNA damaged spermatozoa was significantly different between patients who did and did not establish a pregnancy after IVF.³⁷ DNA damage, which was assessed using the *in situ* nick translation method, did not correlate with fertilization rates or embryo quality. The same study reported a lack of an association between chromomycin A³ fluorescence and any of the IVF outcome parameters.

Tomsu *et al.* measured DNA damage using a modified comet assay 3 to 6 months before couples started IVF treatment cycles. The authors found that the level of sperm DNA damage was correlated with outcome of conventional IVF treatment cycles. Sperm DNA damage was significantly correlated with embryo quality. However, the authors failed to demonstrate any such relationship between sperm DNA status and fertilization.³⁸

The percentage of sperm cells with fragmented DNA (by TUNEL assay) in neat se-

men was negatively associated with the IVF fertilization rate.³⁹ In contrast to other studies, Marchetti *et al.*³⁹ failed to demonstrate a negative correlation between DNA fragmentation and the fertilization rate in prepared sperm.

Katayose *et al.* used diamide-acridine orange staining to detect DNA abnormalities in human sperm. A positive correlation was observed between the fertilization rate after conventional IVF and the green-type increase ratio (percentage of green-pattern sperm after diamide-acridine orange staining/percentage of green-pattern sperm after acridine orange staining).⁴⁰

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.⁴¹ Similar results were reported in the latest study by these authors.⁴² 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.⁶ Depending on the degree of DNA damage, embryo development is affected in later stages and may lead to embryo death in severe cases.

DNA strand breaks in spermatozoa can lead to apoptosis in the spermatozoa. Therefore, measuring levels of apoptosis can assess the degree of DNA strand breaks. Host *et al.* measured levels of apoptosis in spermatozoa and found a significant negative correlation between the proportion of spermatozoa with DNA strand breaks and the proportion of oocytes fertilized after IVF.⁴³

Claassens *et al.* reported that patients with an acridine orange test value of more than 24% had significantly higher oocyte fertilization rates than patients with lower values in IVF and/or GIFT (gamete intra-Fallopian transfer).⁴⁴ However, sperm morphology was found to be a better predictor of fertilization than the acridine orange test.

STUDIES SHOWING NO EFFECT

Defects in chromatin packaging as detected by chromomycin A₃ correlated with fertilization

rates after subzonal insemination (SUZI),⁴⁵ but the authors failed to detect any correlation between chromomycin A₃ positivity and the fertilization rate and embryo viability following IVF. Because chromomycin A₃ competes with protamines for binding sites in sperm DNA, high levels of chromomycin A₃ fluorescence are strongly indicative of a low protamination state.

DNA damage and ICSI

STUDIES SHOWING EFFECT

Correlation with DNA condensation defects.—Sakkas *et al.*^{46, 47} studied the effect of chromatin packaging quality (chromomycin A₃ staining) and the presence of DNA damage (by *in-situ* nick translation method) on fertilization rates after ICSI. They found no difference in the fertilization rate between patients with high and low chromomycin A₃ fluorescence and endogenous nicks. But, unfertilized oocytes from patients with high chromomycin A₃ positivity values and endogenous nicks contained a higher percentage of spermatozoa that remained condensed. This indicates that poor chromatin packaging and damaged DNA may contribute to a failure in the decondensation process.^{46, 47} Razavi *et al.* report significant correlation between DNA damage levels as assessed by CMA3 staining and fertilization outcome post-ICSI.⁴⁸

Correlation with DNA structural integrity and breakage.—Lopes *et al.* studied the relationship between DNA damage and the fertilization rate after ICSI.⁴⁹ DNA damage, which was assessed using the TUNEL technique, was negatively correlated with the ICSI fertilization rate. A fertilization rate of less than 20% was seen in patients with sperm DNA damage of >25%.

Using a modified comet assay in archived sperm, Chan *et al.* found no association between sperm with fragmented DNA and percent fertilization after ICSI. They did, however, report a significant correlation between sperm DNA fragmentation and pregnancy.⁵⁰

Variant-Klun *et al.* studied the effect of

sperm ssDNA (detected by acridine orange) on ICSI outcomes. Spermatozoa with high levels of ssDNA affected the fertilization rate and embryo quality after ICSI. However, the authors failed to find association between the level of spermatozoa with ssDNA and the pregnancy rate and live births rate following ICSI.⁵¹

Morris *et al.* studied the relationship between DNA damage in sperm and IVF and ICSI; they used the comet technique to assess DNA damage.⁵² Percent fertilization, percent cleavage, embryo cell number and embryo grade were lower in the patients who had severe DNA damage than in the patients who had minimal amounts of sperm DNA damage. But, these differences were not statistically different, and no difference was observed in pregnancies and live births. When patients undergoing ICSI were analyzed separately, a significant correlation between DNA damage in sperm and impairment of postfertilization embryo cleavage was noted.

Benchai *et al.*⁵³ reported that 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 embryos that are of good quality.⁵³

Correlation with sperm aneuploidy.—Rubio *et al.*⁵⁴ studied the effect of sperm chromosome abnormalities on ICSI in couples with a history of recurrent miscarriage, repeated implantation failure, previous Down's syndrome or meiotic abnormalities. They found a negative association between chromosomal abnormalities and implantation and pregnancy rates. Miscarriage rates were higher in patients with abnormal sperm FISH results.⁵⁴

In another study,⁵⁵ the male partners of 18 couples undergoing ICSI were evaluated for sperm aneuploidies and their association with successful outcome after ICSI. The authors noted that only 2 patients (29%) in the group of men who were able to impregnate their partners had a higher frequency of sperm aneuploidies than the controls. In contrast, 10

patients (91%) in the group of men who were not able to impregnate their wives had a higher frequency of sperm aneuploidies than the controls.⁵⁵ It is important to note that one patient was able to impregnate his wife despite having sperm with a high frequency of aneuploidies. However, in this study, the patients' mean age was higher than that of the control group.

Burrello *et al.* studied the impact of sperm aneuploidy on ICSI outcome in patients with male factor infertility.⁵⁶ They reported that patients with sperm that had a high frequency of aneuploidy (>1.55%) had significantly lower pregnancy and implantation rates. The fertilization and embryo cleavage rates were similar between patients with a low or high frequency of sperm aneuploidy.

Storeng *et al.* studied the association between sex chromosome abnormalities and fertilization rates after IVF and ICSI.⁵⁷ The incidence of sex chromosomal abnormalities was higher in the patients undergoing ICSI than in those undergoing IVF. The authors attributed this finding to the fact that there were more couples with female infertility in the IVF program than those undergoing ICSI. This study emphasizes the importance of studying the effect of genomic abnormalities in a uniform patient population rather than unselected patients seeking assisted conception. Similarly, the authors of a single case report found that the male partner of an infertile couple who was referred for ICSI had a high percentage of sperm with chromosome abnormalities, which included sex chromosome aneuploidy.⁵⁸

Pang *et al.* studied 9 patients with oligoasthenoteratozoospermia who were part of infertile couples being referred for ICSI. The authors found that all 9 men had a high frequency of sperm aneuploidy. These patients were not able to initiate or sustain a pregnancy in their partners.⁵⁹ In another study, the authors reported a 70% fertilization rate and 20% pregnancy rate in men with oligoasthenoteratozoospermia who were referred for ICSI with their partner; these men also had a higher aneuploidy rate than the study controls.⁶⁰

Other researchers reported that the zona pellucida might be able to select against aneu-

ploid spermatozoa in natural fertilization. They showed that spermatozoa that were bound to the zona pellucida had a low frequency of aneuploidy.⁶¹ A significant correlation was noted between the presence of aneuploidy in sperm and the fertilization rate after ICSI. The pregnancy rate dropped when semen samples contained more diploid than haploid spermatozoa.

STUDIES SHOWING NO EFFECT

Correlation with DNA condensation defects.—Decondensation of sperm DNA is an important step that enables sperm to fertilize an oocyte. 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. They used the aniline blue stain technique and compared the rates between patients with 0-29% and more than 29% positive aniline blue stained spermatozoa.⁶²

Intra cytoplasmic sperm injection can be performed using spermatozoa extracted from testicular biopsy. The degree of sperm chromatin condensation in these spermatozoa and their ability to achieve fertilization and pregnancy were compared with those of freshly ejaculated spermatozoa.⁶³ There was a significant difference in chromatin condensation (as assessed by aniline blue staining) between the ejaculated spermatozoa and sperm extracted from a testis biopsy. However, no such difference was seen in fertilization and pregnancy rates between these 2 groups. According to the authors, these results suggest that there is no relationship between the percentage of chromatin condensation and fertilization and pregnancy rates following ICSI.

The ability of sperm DNA to undergo decondensation *in vitro* and its association with the fertilization rate after ICSI was studied by Hammedah *et al.* No correlation was found between the mean percentage of spermatozoa undergoing chromatin decondensation *in vitro* and fertilization rates.⁶⁴

Correlation with DNA structural integrity and breakage.—Angelopoulos *et al.* assessed whether the degree of sperm nuclear matur-

ity (as assessed with acridine orange staining) can predict fertilization or pregnancy outcomes after standard IVF and ICSI. There was no significant difference in the degree of acridine orange staining between the patients with or without successful fertilization or pregnancy.⁶⁵

Host *et al.* found no association between DNA strand breaks (as measured by apoptosis levels) in spermatozoa with proportion of oocytes fertilized after ICSI.⁴³ Henkel *et al.* found no correlation between sperm DNA fragmentation (as measured by apoptosis levels) and outcome parameters in patients undergoing ICSI.⁴¹ However, the TUNEL-positive group with high levels of sperm DNA fragmentation (>24.3%) had a lower rate of pregnancy after ICSI than the TUNEL-negative group. A recent study also showed no relationship between SCSA parameters and ART outcome after ICSI.⁶⁶

Correlation with sperm aneuploidy.—In contrast to the recent studies, Yoshida *et al.* found that fertilization rates were higher in men with structural autosomal abnormalities (n=6) compared to men without structural autosomal abnormalities (n=31). There was no significant difference in the rate of embryo cleavage between 2 groups. They concluded that autosomal structural abnormalities do not influence fertilization and early embryonic development after ICSI.⁶⁷

DNA damage and IVF and ICSI

STUDIES SHOWING EFFECT

Saleh *et al.* reported that the 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); $p=0.001$].²⁶

Using the SCSA technique, Larson *et al.* predicted pregnancy outcome following IVF and ICSI.⁶⁸ They reported that SCSA was predictive of negative pregnancy outcome when the spermatozoa in the neat semen showed acid-induced DNA denaturation of $\geq 27\%$ DFI. However, the authors found no relationship

between the SCSA parameters and normal fertilization and early embryo development. The findings of this study were confirmed in a large study consisting of 89 couples undergoing ARTs.⁶⁹

STUDIES SHOWING NO EFFECT

The predictive value of DFI >27% by SCSA in predicting assisted reproduction success appears to be better with ICSI than IVF. Authors could not demonstrate any difference in clinical pregnancy in patients undergoing IVF and ICSI when the DFI was >27% and ≤27%.²⁷ This study found, contrary to the previous reports,⁶⁸ that a DFI >27% is incompatible with pregnancy and delivery after IVF or ICSI.

Unlike other studies, Colombero *et al.* reported that the fertilization rate and pregnancy outcome after IVF and ICSI were not affected by increased frequencies of chromosomal abnormalities as detected by the FISH technique.⁷⁰ These authors failed to find any increase in pregnancy losses or the occurrence of neonatal malformations in patients with increased frequency of sperm aneuploidy. But, in this study, the authors used a relatively small number of spermatozoa (approximately 1 725 per sample) to assess chromosomal abnormalities.

Discussion

The role that standard semen parameters play in the evaluation of sperm quality has become debatable with the advent of ART procedures. These procedures bypass natural selection mechanisms, which increases the chance that sperm with abnormal genomic material will fertilize an oocyte.⁷¹ Extensive data exists on the relationship between sperm DNA damage and outcomes after ARTs. Most of the studies have shown a significant negative association or negative trend with fertility outcome parameters after ARTs. Some studies have found no correlation between levels of DNA damage and fertilization rates but rather an association between DNA damage and postfertilization embryo development. This may relate to the

degree of DNA damage and the importance of the abnormal genomic material in these processes.

Many 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 ICSI studies found 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 the study population.

Most of the studies that we reviewed lack a well-defined study population. Studies that included couples undergoing ARTs included a broad spectrum of patients with female factor or male factor infertility or infertility of unknown etiology. The effect of sperm DNA damage on ARTs will become more clear when studies enroll patients with male factor infertility only rather than a mix of patients with different causes of infertility. Idiopathic infertility and female factor infertility should be analyzed separately in infertile couples with normal sperm parameters to determine if DNA damage is a contributing factor. Studying sperm DNA damage in patients with no obvious male factor cause may help support the belief that DNA status can be an independent measure of sperm quality.

Another question that needs to be studied is whether the success of ARTs are correlated with neat sperm or prepared sperm DNA quality. The method of sperm preparation is another variable in these studies. Damage in neat sperm may accurately reflect DNA quality of men, which is important in natural conception. But, sperm preparation procedures are used in conjunction with ARTs to enrich spermatozoa.⁷² The yield of spermatozoa with mature DNA is not uniform with different sperm preparation methods. Because most centers use some form of sperm preparation method, studies should correlate assisted reproduction outcome with prepared

sperm DNA quality rather than neat sperm DNA quality. Marchetti *et al.*³⁹ have already reported that the fertilization rate is negatively correlated with the percentage of sperm cells with fragmented DNA in neat semen but not in prepared sperm suspensions. Thus, studies may initially need to use both neat and prepared spermatozoa to check for DNA damage and its association with assisted reproduction success for a clear understanding of this subject.

Despite these drawbacks in the existing literature, there is enough evidence now to show that DNA damage influences the fertility outcome after ARTs. Thus, it is prudent to check for DNA damage in infertile patients undergoing ARTs. It is important to standardize the protocols used to detect chromosomal abnormalities and DNA damage so that they can be used routinely in laboratories.² There is also a need for studies to 1) implement good quality control, 2) use a sufficiently large number of samples, and 3) employ common methods of assessing DNA damage. Finally, researchers should attempt to use a uniform study population (*e.g.*, couples with male factor infertility only), which will provide more confirmatory evidence regarding the role of sperm DNA damage in fertility.

Acknowledgements.—The authors wish to thank the Glickman Urological Institute of the Cleveland Clinic Foundation for research support and R. Verdi for secretarial support.

Riassunto

Effetti del danno al DNA spermatico sull'esito della fecondazione assistita: una review

La qualità del DNA spermatico è molto importante per il mantenimento delle potenzialità riproduttive dell'uomo. Il DNA spermatico è resistente a molti tipi di insulto che si verificano durante il suo viaggio dal testicolo al momento in cui raggiunge l'ovocita per la fecondazione. Durante il concepimento per via naturale, si verifica un processo di selezione che consente solamente agli spermatozoi con DNA intatto di fecondare l'ovocita. Quando vengono utilizzate le tecniche di fecondazione assistita, questo processo viene scavalcato in parte o nella sua totalità. Di conseguenza, è possibile che uno spermatozoo con DNA danneggiato fecondi l'ovocita. Il danno al DNA spermatico condiziona negativamente la feconda-

zione assistita e quella naturale. Il DNA spermatico viene sempre più spesso utilizzato come misura indipendente della qualità dello sperma, dal momento che può fornire informazioni di tipo diagnostico e prognostico più accurate rispetto ai parametri standard dello sperma. La presente review riassume i dati attualmente a disposizione circa il ruolo del danno al DNA spermatico nella fecondazione assistita. Da questi dati emergono 2 punti fondamentali: 1) gli uomini con DNA spermatico danneggiato presentano una ridotta capacità di generare figli; 2) gli spermatozoi che presentano anomalie al DNA sono in grado di fecondare l'ovocita e di generare un embrione vitale. Gli uomini con problemi di fertilità, che vogliono fare ricorso alle tecniche di fecondazione assistita insieme alla loro partner, devono sottoporre il proprio sperma all'analisi dell'integrità del DNA. I risultati di questa analisi possono essere utilizzati per informare la coppia circa la possibilità di generare embrioni vitali e di trasmettere anomalie genetiche ai figli.

Parole chiave: DNA - Spermatozoi - Infertilità - Riproduzione assistita - Fertilizzazione *in vitro* - Gravidanza.

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