Clinical value of DNA fragmentation evaluation tests under ART treatments

Yardımcı Üreme Teknikleri tedavilerinde sperm DNA fragmentasyonu değerlendirilmesinin klinik etkileri

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Abstract

Male reproductive health has been under scrutiny recently. Many studies in the literature have concluded that semen quality is declining and that the incidence of testicular cancers is increasing. The reason for this change has been attributed to damage in sperm chromatin. During in vivo reproduction, the natural selection process ensures that only a spermatozoon with normal genomic material can fertilize an oocyte. However, the assisted reproduction technique (ART) is our selection process, leading to the possibility that abnormal spermatozoa could be used to fertilize an oocyte. We could avoid this by quantifying the amount and type of genomic damage in sperm using well-accepted laboratory methods. The sperm deoxyribonucleic acid (DNA) integrity is important for success of natural or assisted fertilization as well as normal development of the embryo, fetus and child. Intra cytoplasmic sperm injection (ICSI) is bypassing natural sperm selection mechanisms, which increases the risk of transmitting damaged DNA. The significance of required investigations and multiple techniques is that they could evaluate DNA defects in human spermatozoa. The ability of these techniques to accurately estimate sperm DNA damage depends on many technical and biological aspects. The aim of this review is to evaluate the most commonly used methods.

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Key words: Tunel, Comet, Acridine Orange staining technique (AOT), Sperm Chromatin Dispersion (SCD), Sperm Chromatin structure (SCSA)

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Özet

Erkek üreme sağlığıyla ilgili son zamanlardaki çalışmalar semen kalitesi ve sıklığı artan testiküler kanser vakaları üzerinde yoğunlaşmaktadır. Normal üreme şartlarında spermlerin doğal seleksiyonu söz konusu olmakta ve normal genetik yapıdaki spermlerle oositlerin fertilizasyonu gerçekleşmektedir. Ancak yardımcı üreme teknikleri ile gerçekleştirilen tedavilerde sperm seçimi semen kalitesine bağlı olarak da anormal spermlerle geçekleştirilebilmektedir. Bu durumlarda kabul görmüş birtakım laboratuvar testleri uygulayarak hasarlı genetik yapıdaki spermlerden korunabilmek mümkün olabilmektedir. Sperm deoksiribonükleik asid (DNA) bütünlüğü; doğal ve yardımcı yöntemlerle fertilizasyonun başarısı aynı zamanda embriyo, fetüs ve çocuğun normal gelişimi için önemlidir. İntrasitoplazmik sperm enjeksiyonunda (ICSI) doğal sperm seçim mekanizmaları devre dışı kalmakta, bu da hasarlı DNA'nın transfer riskini artırmaktadır. Bu derlemede sperm DNA fragmantasyonu oranı belirlemede en sık kullanılan testler değerlendirilmektedir. (J Turkish-German Gynecol Assoc 2012; 13: 270-4)

Anahtar kelimeler: Tunel, Comet, Acridine orange boyama tekniği (AOT), sperm kromatin dağılımı testi (SCD), sperm kromatin yapı testi (SCSA)

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Introduction

Sperm quality is ferquently used as an indirect measure of male infertility. The parameters that have been used historically as indicators of male infertility potential include sperm count, motility and morphology, all of which are evaluated in fertility clinics as a part of routine semen analyses (1). Assisted reproductive techniques such as conventional in vitro fertilization (IVF) and intra cytoplasmic sperm injection (ICSI) allow couples whose sperm parameters are impaired to achieve a pregnancy. Among these factors which are involved in the failure to obtain embryos and pregnancies, the impaired sperm genom is frequently incriminated (2, 3). To assist in the risk assesment of ICSI, it would be appropriate to develop methods to measure deoxyribonucleic acid (DNA) damage in the sperm and to correlate this with biological outcomes. DNA abnormalities in sperm are well documented. Cytogenetic analysis of sperm chromosomes has demonstrated sperm aneuploidy, which, although low in frequency, is assosciated with infertility and adverse pregnancy outcome (4, 5). Several techniques and investigations are proposed in order to study these abnormalities. Those which are currently used are; the Tunel test, which allows the evaluation of the sperm DNA fragmentation (6, 7), the Comet test, which represents another way of evaluating the DNA integrity (8, 9) and DNA staining by acridine orange (AO), which differenti-

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ates between single and double stranded DNA based on their reactive colors under fluorescence and thus allows the degree of DNA denaturation to be evaluated (3). Other tests identify the packaging defects of sperm chromatin: aniline blue staining, toluidine blue staining, and chromomycin A3 staining (10).

Sperm DNA and Abnormalities

Deoxyribonucleic acid of sperm is organized in a special way that keeps the nuclear chromatin compact and stable (11). This DNA not only permits the tightly packaged genetic information to be transferred to the oocyte but also ensures that the DNA is delivered in a physical and chemical form that allows the developing embryo to easily access the genetic knowledge. Fertile and normal sperm have stable DNA, which is able to undergo decondensation at the same time in the fertilization process and transmit the DNA without defects.

Defective genomic material in sperm may cause the formationof condensation or nuclear maturity defects, DNA breaks, DNA integrity defects, or sperm chromosomal aneuploidies (12). The causes of these defects have been attributed to disease, drug use, high fever, more than normal testicular temperature, smoking, and advanced age. DNA damage's molecular mechanism in these different conditions is under intense investigation. The most important mechanisms for sperm DNA damage are abnormal chromatin packaging, reactive oxygen species (ROS) (13), and apoptosis (14, 15). It is likely that multiple mechanisms are involved, based on the clinical diagnosis responsible for DNA damage.

Comet Assay

Comet assay uses single cell gel electrophoresis (SCGE) to analyze DNA fragmentation in individual cells, was first introduced in 1984 by Ostling and Johanson (16) who used neutral buffer conditions to study double-stranded DNA breaks (17). This assay is extensively used in somatic cells to measure genotoxic damage, especially single and double strands breaks and was originally applied to sperm by Singh (18). The Comet assay may therefore be used to study single or double stranded DNA breaks in somatic cells or germ cells and is useful because it allows for the distinction between the different kinds of DNA fragmentation necrotic and appoptotic cells. Appoptotic cells produce teardrop shape comets during electrophoresis (19). The shape is due to the migration and accumulation of the short DNA fragments and the intensity of the tail represents the amount of DNA fragments present (20).

Tunel Test

This test was originally described by Garvrieli, Sherman, and Ben-Sasson in 1992 (21). Tunel has become one of the main methods for detecting apoptotic programmed cell death. However, there has been a debate about its accuracy, due to problems in the original assay, which caused necrotic cells to be inappropriately labeled as an apoptosis (22). The method has subsequently been improved dramatically to identify only cells in the last phase of apoptosis (23, 24). New methods incorporate the dUTPs modified by fluorophores or haptens, including biotin or bromine, which can be detected directly in the case of a fluorescently-modified nucleotide (fluorescein-dUTP), or indirectly with streptavidin or antibodies, if biotin-dUTP or BrdUTP are used, respectively.

The TUNEL assay detects both single- and double-stranded DNA breaks by labeling the free 39-OH terminus with modified nucleotides in an enzymatic reaction with terminal deoxynucleotidyl transferase (TdT) and can be analyzed microscopically or by using flow cytometry.

Acridine orange staining technique (AOT), sperm chromatin dispersion (SCD) and sperm chromatin structure (SCSA) tests

The acridine orange staining technique (AOT) is a simple microscopic procedure based on the same principle as the sperm chromatin structure assay (SCSA) but indistinct colours, rapid fading of fluorescence, and heterogeneous staining of slides makes AOT a test of questionable value in clinical practice (25). The SCSA is fluorescence activated cell sorter test, measures the susceptibility of sperm DNA heat or acid induced DNA denaturation in situ followed by staining with acridine orange (26).

Recently, a new method, the sperm chromatin dispersion test (SCD), was introduced for evaluating sperm DNA fragmentation (27-37). The SCD test is based on the principle that sperm with fragmented DNA fails to produce the characteristic halo of dispersed DNA loops that is observed in sperm with non-fragmented DNA following acid denaturation and removal of nuclear proteins.

Evaluation of tests used under ART treatments

Several authors were included to diagnose with the tests results of their differently based researches.

Table 1 shows that some authors had reported a significant relationship between sperm DNA fragmentation index and pregnancy rate (3, 32, 34-37). On the other hand, many others revealed no significant relationship (9, 28-31). However, these controversial results may be attributed to different principles of the techniques of the analytical methods used, as represented in Table 2. Each assay method has their advantages and disadvantages.

Conclusion

Sperm DNA integrity is associated with male infertility potential in vivo and in vitro. There are increased levels of fragmented sperm DNA in a high percentage <40% of men presenting as clinically subfertile. Especially semen with a high percentage of damaged spermatozoa has a very low potential for natural fertility. DNA damage in sperm does not preclude IVF as there is still a chance that samples in which sperm have damaged DNA can be used to achieve a pregnancy. ART studies mentioned that the reproductive parameters that could be affected by the integrity of the DNA in ejaculated spermatozoa include fertilization, blastocyst development and pregnancy rates. In fact, pregnancy rates using conventional IVF and ICSI treatments are significantly reduced in couples with a high percentage of sperm with DNA damage.

All literature shows that sperm DNA damage influences the fertility outcome to different degrees, but there is no consensus

Table 1. The statistical relationship between sperm DNA fragmentation index (DFI %) and pregnancy rate with different analytical tests under ART treatments as reported by some authors

Authors	DFI %	ART Procedure	Patient #	Statistical Results	Analysis
Chohan et al. (28)	<30	IVF or ICSI	52	Not Significant	SCSA, Tunel, SCD
Larson et al. (3)	<27	ICSI	21	Significant	SCSA
Check et al. (29)	<30	ICSI	106	Not Significant	SCSA
Morris et al. (9)	Low DNA Damage	IVF or ICSI	52	Not Significant	Comet
Bungum et al. (30)	<27	IVF	109	Not Significant	SCSA
Bungum et al. (30)	>27	IVF-ICSI	66	Not Significant	SCSA
Larson-Cook et al. (31)	<27	IVF	55	Not Significant	SCSA
Larson-Cook et al. (31)	<27	ICSI	26	Not Significant	SCSA
Virro et al. (32)	<30	IVF	249	Significant	SCSA
Spano et al. (33)	<30	In-vivo	215	No Result	SCSA
Everson et al. (34)	<30	In-vivo	147	Significant	SCSA
Henkel et al. (35)	<36.5	IVF	208	Significant	Tunel
Henkel et al. (36)	<36.5	IVF	167	Significant	Tunel
Caglar et al. (37)	> 4	ICSI	56	Significant	Comet
Caglar et al. (37)	> 4	ICSI	56	Significant	Tunel

DFI: Deoxyribonucleic acid Fragmentation Index, ART: Assisted Reproductive Techniques, SCSA: Sperm chromatin structure assay, SCD: Sperm chromatin dispersion test, IVF: In vitro fertilization, ICSI: Intra Cytoplasmic Sperm Injection, DNA: Deoxyribonucleic acid

Table 2. Evaluation of different analytical tests (principles, detection method, advantages and disadvantages) used in ART treatments

Assay	Principle	Detection method	Adventages	Disadvantages
Tunel	Single & double strand DNA breaks	Fluorescence microscopy, Flow cytometry	Clinically significant high sensivity and specificity large number of spermatozoa counted by flow cytometry	Special equipment, more expensive
Comet	Single & double strand DNA breaks or only double strand DNA breaks	Fluorescence microscopy	Related to Tunel assay, cheap, high sensitivity qualification of DNA damage in individual cells, evaluation of different type of DNA damage	Special equipment and experienced observer
Acridine Orange	Differentiates between single & double stranded DNA	Fluorescence microscopy	Easy to perform, cheap	Special equipment distiction between differenty labelled spermatozoa, not always easy
Sperm Cromatin Decondensation	Evaluation of DNA decondensation halo	Fluorescence microscopy, Optical microscopy	Easy to perform, cheap	Clinical relevance not yet proven
Sperm cromatin stracture assay	Susceptibility of DNA to acid denaturation	Flow cytometry	Clinically significant high sensivity and specificity large number of spermatozoa counted by flow cytometry, unbiased quantitative assessment of DNA bound acridine orange	Special equipment, more expensive

DNA: Deoxyribonucleic acid

on the technique that should be used to measure sperm DNA in subfertile patients. The methods used to detect sperm DNA damage should be standardized to allow comparison among different studies and to permit routine use of tests in clinical laboratories. The results of degrees of DNA damage could give better decision facilitation to physicians on infertile couples about their chances of having a live birth. New researchs aim to identify the type of DNA defects that affect fertility regardless of the quantity of damaged DNA and to identify and isolate spermatozoa with intact DNA for ART. The TUNEL, AOT, and SCD are simple, less expensive procedures and can be performed in a short period of time to assess the levels of DNA fragmentation in sperm from infertile men and donors of known fertility.

Conflict of interest

No conflict of interest was declared by the authors.

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