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Review

Sperm DNA and detection of DNA fragmentations in sperm

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ABSTRACT

The questionable effectiveness of routine sperm parameters in determining male factor infertility problems and increasing the success rates of assisted reproductive techniques have led to the investigation of more detailed sperm parameters that could affect the male fertility and reproduction. Thus, the effects of different sperm parameters such as sperm DNA integrity was started to be investigated thanks to the previously described methods such as single cell gel electrophoresis (COMET) assay, sperm chromatin structure assay (SCSA), acridine orange test (AOT), terminal deoxynucleotidyl transferase-mediated deoxyuridine (TdT) triphosphate (dUTP) nick end labeling (TUNEL) assay and sperm chromatin dispersion (SCD) test. However, studying on sperm DNA might be very complex because the sperm DNA differs from the somatic cell DNA with its unique structure. Also, the sperm DNA undergoes many changes during spermatogenesis and it is condensed by being packaged tightly with different types and numbers of protamines in different species. Despite all these difficulties, these methods provide important information about the reasons and consequences of DNA damages in sperm and the effects of these damages on reproduction.

Keywords: COMET; DNA fragmentation; SCD test; sperm; SCSA; TUNEL.

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Introduction

Sperm is the male gamete cell which can move and has the ability of capacitation, penetration and fertilization. The ultimate aim of sperm is to maintain the generation by transferring the male genetic material into the ovum, the female gamete cell.

Routine semen examinations are widely used to predict the fertilization ability of sperm. Routine semen parameters consist of sperm concentration, motility, live sperm ratio and morphological examination. However, the previous studies conducted in humans have demonstrated that it is not always possible to achieve an accurate decision about male fertility according to the results of routine semen examinations[1,2] and when all the routine sperm parameters are normal, sperm population of an ejaculate may be subfertile or infertile.[2] Therefore, the effects of more detailed parameters such as deoxyribonucleic acid (DNA) integrity of sperm on the fertility are considered. Previous studies have indicated that percentage of DNA damaged sperm in the sperm population of the individuals could affect the fertilization capacity of their sperm, the qualities of their embryos and pregnancy outcomes achieved after assisted reproductive techniques.[1,3] Thus, it is considered that detection of sperm DNA integrity might provide complementary data to predict fertilization capacity of sperm, quality of embryos obtained from these sperms and success rate of assisted reproductive techniques such as *in vitro* fertilization and intracytoplasmic sperm injection. The detection of DNA damaged sperm is not only important to find out their harmful effects on reproduction in mammals but also to take preventive measures, and understand the causes and consequences of DNA damage in sperm.

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Sperm DNA

The DNA of mammalian sperm is the mostly known compact eukaryotic DNA which is packaged six times more tightly than the tightly packaged mitotic chromosomes of somatic cells.[4] For example, the sperm nucleus of mouse is compacted into a volume that is approximately 20 times smaller than the liver cell nucleus.[5,6] In the mammals, the DNA of sperm is situated as anchored to a single structure which is called the nuclear annulus.[7,8] It is believed that the nuclear annulus is important in the organization of sperm DNA.[8] The previous studies conducted on chromatin condensation during spermiogenesis indicated that this process starts from the anterior tip of sperm head and progresses towards the tail.[9,10] In the process of chromatin condensation, after histones are restructured by transition proteins, they are reconstructed again to finalize their shape by protamines which are DNA-binding proteins of sperm.[11] The protamines are half size of histones and the majority of the amino acids in protamines are arginine.[12] The sperm of different species contain varying numbers of different types of protamines with different amino acid sequences. It was reported that the sperm nuclei of bulls,[13] rams[14] and rats[15] contained a single type of protamine, sperm nuclei of mice[16] and humans[15,17] contained two types of protamines, and sperm nuclei of some fish species[18] contained three different types of protamines.[12] Contrary to the sperm of some species such as bull that has a single type of protamine, the sperm of other species like human consist of two different types of protamines which contain less cysteine.[19] This situation leads to formation of fewer disulfide bonds in the sperm nuclei which contain two different types of protamines and being less stable than those which contain a single type of protamine.[19]

DNA fragmentation in sperm

It is known that DNA damage occurs in sperm due to various reasons. The most common cause of DNA damage in sperm is oxidative stress. Oxidative stress occurs due to the imbalance between the production of reactive oxygen species and antioxidant defense system.[20] The fact that spermatozoon membrane contains high amounts of polyunsaturated fatty acid and its cytoplasm has inadequate antioxidant capacity make spermatozoa highly vulnerable to the attacks of reactive oxygen species and lipid peroxidation.[21,22] For this reason, many different factors can cause oxidative stress and DNA damage in spermatozoa. For example, it was reported that heat stress,[23] cryopreservation of sperm[24,25] and chilling of sperm[26] cause DNA damage in the sperm. It is known that smoking, cancer therapies, varicocele, and cancer can lead to DNA damage in the human sperm.[27] Also, DNA damage can occur in germ cells during spermatogenesis and some DNA damaged germ cells are eliminated via apoptosis.[28] Thus, the disturbances related to the apoptotic mechanism might lead to DNA damaged sperm production.

Detection of DNA fragmentation in sperm

A number of different methods have been described to determine the DNA damaged sperm rate and the degree of these damages. The primary methods are as follows; Single Cell Gel Electrophoresis (COMET) Assay, Sperm Chromatin Structure Assay (SCSA), Acridine Orange Test (AOT), Terminal Deoxynucleotidyl Transferase-Mediated Deoxyuridine (TdT) Triphosphate (dUTP) Nick End Labeling (TUNEL) Assay and Sperm Chromatin Dispersion (SCD) Test.

Single cell gel electrophoresis

The COMET assay was first described by Ostling and Johanson in 1984.[29] They detected double-stranded DNA fragments in somatic cells using neutral lysis and electrophoresis solutions. Neutral COMET assay was evolved later on by two different groups using alkaline solutions.[30,31] Alkaline COMET assay enabled identification of the lower levels of DNA fragments and both single and double strand DNA breaks.[30] In general, COMET assays are based on the evaluation of the comet image which is formed when the DNA, which is revealed after embedding the certain number of sperm into the agar and lysing their cellular proteins and membranes using detergent and high density salt solutions, is exposed to electrophoresis and the existing DNA fragments move further than the main DNA. The basic steps for this technique are summarized below.

The process of embedding of spermatozoa into the agar: At this stage, the researchers covered the slide which had been previously coated with normal melting agar (NMA) with a low melting agar (LMA)-sperm solution which contained a certain number of spermatozoa.[32,33] However, it was observed that some researchers preferred directly spreading the agar-cell solution on the frosted microscope slide or gelbond.[34,35] The aim of using normal melting agar, frosted microscope slide or gelbond as a first layer might be to prevent possible loss of the cell-agar mixture during the lysis and electrophoresis procedures.

The lysis process: The aim of this process to lyse sperm membranes and reveal the sperm DNA during the incubation period in lysis solutions. Several lysis solutions including variety of chemicals have been used for this process at different time intervals in various species.[33,36-39]

The process of electrophoresis: In this process, after the incubation of revealed sperm DNA in neutral or alkaline electrophoresis solution, the revealed sperm DNA is exposed to the electrophoresis in neutral or alkaline conditions. Different electrophoresis solutions, variety of electric powers and application times were used in the electrophoresis process in previous studies.[34,36-39]

Assessment of comet images: In this process, the slides are incubated in the neutralization solution, air dried and stained with DNA specific stains. The stained comet images are assessed subjectively under fluorescence microscope or by computer-assisted COMET assay analysis program linked to fluorescence microscope. COMET assay analysis programs or different computer-assisted image analysis programs were used for the assessment of the comet images in previous studies.[33,38,40,41] The COMET assay specific analysis programs might be the most objective method to be used for the assessment of comet images. Because it is possible to obtain many different parameters from these programs such as tail DNA (%), tail length (μm) and Olive tail moment which show the degree of the DNA damage in individual cells automatically.

Sperm chromatin structure assay

SCSA was described for the first time in 1980.[42] SCSA is based on the principle that DNA damaged sperm are more vulnerable to heat or acid denaturation compared to the intact sperm.[42,43] The denatured DNA damaged sperm are stained red while the intact sperm turn into green when exposed to acid and acridine orange stain.[44] The stained sperm are analyzed by flow cytometry and DNA fragmentation index (DFI) is calculated to determine DNA damaged sperm rate. The greatest advantage of SCSA is that a large number of cells and 1024 discrete channels of red and green fluorescence could be evaluated by flow cytometry.[45,46]

Acridine orange test

Generally, AOT is similar to SCSA but the DNA damaged and intact sperm are classified subjectively under florescence microscope in AOT. When the intact sperm emit green florescence, DNA damaged sperm emit red florescence.[44] After the evaluation of spermazoa according to their color, DNA damaged sperm rate are detected. AOT is simpler and cheap test compared to the SCSA. But, the evaluation of limited number of spermatozoa, the quick loss of florescence emissions under florescence microscope and limited classification of florescence emissions between green and red with naked eye are the disadvantages of AOT.[45-47]

Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling assay

TUNEL test is based on detection of single or double stranded DNA fragments marked by dUTP owing to a reaction which are catalyzed by the TdT enzyme. The TUNEL test generally consists of respectively fixation and permeabilization of sperm, marking of DNA fragments, staining and evaluation of sperm stages. In the evaluation process, the sperm can be assessed subjectively under light and fluorescence microscope or by flow cytometry.[48-50] The TUNEL assay can be also used to identify the apoptotic cells in the tissues during histological examinations.[51,52]

Sperm chromatin dispersion test

In classical SCD test, the slides, which are covered with sperm-agar solution, are prepared similarly as in COMET assay procedure. Then, these sperm samples are incubated respectively in acid denaturation solution to reveal fragmented DNA in sperm, in neutralizing-lysing solutions to remove cellular proteins, and in washing and ethanol solutions to wash and dehydrate the samples.[53] The air-dried sperm samples are stained with Diff-Quik stain or DNA specific stains and the sperm samples are evaluated under light or fluorescence microscope.[53] In classical SCD test, while the sperm which produced halo were regarded as intact, the sperm which had no halo were regarded as DNA damaged.[53-55] Contrary to the classical version of SCD test, the sperm which produced halo are regarded as DNA damaged; the sperm which had no halo are regarded as intact in modified commercial version of the SCD test.[55-58]

Comparison of tests used to detect DNA damage in spermatozoa

In humans, SCSA, TUNEL and SCD tests have been found quite effective in detecting DNA damage in sperm contrary to AOT.[46] Although SCSA and AOT have same experimental principles; the reason of different results obtained from these tests might be a consequence of different evaluation procedures.[46] Both COMET assay and SCSA were found to be more sensitive to distinguish and detect DNA damage in mouse spermatozoa which were collected from caput or cauda of epididymidis compared to SCD test and TUNEL assay.[59] Sperm chromatine condensation and the formation of disulphide bridges between protamines maintain during transport of sperm through epididymis. Thus, the sensitivity of these tests might be dependent on sperm maturity.[59]

In conclusion, the previous studies showed that all these tests can be applied to determine the preventive measures, causes and consequences related to DNA damage in sperm. Also, these tests provide complementary information to predict fertilization capacity of sperm, success rates of assisted reproductive techniques and qualities of embryos which are obtained from this sperm. However, the sensitivity of these tests might change dependent on the species, maturational stages of sperm and different evaluation methods. The widespread usage of flow cytometry and computer assisted analysis programs can contribute standardization of the results obtained from different tests. Also evaluation of oxidative stress parameters in sperm might be beneficial to support results of these tests.

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