Review

DNA damage of human spermatozoa in assisted reproduction: origins, diagnosis, impacts and safety

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Abstract

Sperm DNA contributes half the offspring's genomic material and abnormal DNA can lead to derangements in the reproductive process. Normal sperm genetic material is required for successful fertilization, as well as for further embryo and fetal development that will result in a healthy child. Thus, the damage to sperm DNA is critical in assisted reproductive techniques which are increasingly used to treat infertile couples. There has been improving data about the effects of human sperm DNA damage or fragmentation. As well, increasing knowledge concerning the effects of DNA damage on embryo and fetal development has been attained. This review aims to summarize the present knowledge on the impact of human sperm cell DNA damage on male infertility and outcome in the context of safety.

Keywords: *apoptosis, assisted reproduction, DNA damage, safety, sperm, outcome*

Introduction

Assisted reproduction techniques have currently undertaken a major role in treating infertility. Success rates depend on a variety of factors most important being the structural and functional integrity of the gametes used. Therefore, the exact nature of infertility and its cause or pathogenesis should be considered before initiation of treatment. Unidentified factors may adversely affect the end results and add to the financial, social and emotional problems of the patients.

The positive relationship between poor sperm parameters and DNA damage in human spermatozoa points to inherent problems in spermatogenesis in specific patients. Various hypotheses have been proposed as to the molecular mechanism of sperm DNA damage. The most important ones are abnormal chromatin packaging, oxidative stress and apoptosis (Sakkas *et al.*, 1999).

Semen samples that contain high levels of DNA damage are often associated with decreased fertilization rates or embryo cleavage after IVF and intracytoplasmic sperm injection (ICSI) and may be linked to early embryo death. Although the most normal appearing and motile spermatozoa are selected, there is always a chance that spermatozoa containing varying degrees of DNA damage may be used. Thus, one of the main disadvantages of using assisted reproduction is the bypass of the natural selection barriers that are present throughout the female reproductive tract until spermatozoa enters the oocyte (Chandley and Hargreave, 1996): spermatozoa with abnormal genomic material can reach the genetic material of the oocyte with minimal (IVF) or no effort (ICSI) at all. The miscarriage rate is higher after ICSI, which possibly reflects the fact that genomically compromised spermatozoa are sometimes used and lead to

irreparable DNA damage in the embryo (Carrell *et al.*, 2003; Agarwal and Said, 2005).

This review aims to summarize the present knowledge on the impact of human sperm cell DNA damage, in the context of the different damage origins, on outcome and prognosis of male infertility, as well as the safety of using DNA-damaged human spermatozoa in assisted reproduction.

Origins of DNA damage of human sperm

Spermatogenesis is a complex process of male germ cell proliferation and maturation from diploid spermatogonia through meiosis to mature haploid spermatozoa (de Kretser *et al.*, 1998). However, damage of sperm DNA or its chromatin structure can occur at any step of whole spermatogenesis (Erenpreiss *et al.*, 2006). The positive relationship between poor sperm parameters and DNA damage in mature spermatozoa points to inherent problems in spermatogenesis in specific patients (Irvine *et al.*, 2000; Agarwal and Said, 2005). Three theories have been proposed to explain DNA anomalies in the ejaculated human spermatozoa. The first theory supports that DNA damage in mature spermatozoa is associated with poor chromatin packaging or abnormal packing due to underprotamination which results in the presence of endogenous nicks in DNA (Manicardi *et al.*, 1995; Sakkas *et al.*, 1999). The second theory proposes that the presence of endogenous nicks is characteristic of programmed cell death aiming to the functional elimination of possibly defective germ cells from the genetic pool (Sakkas *et al.*, 1999). Recent models of apoptosis include receptor-mediated pathways and intrinsic triggered apoptosis, as well as cytotoxic or stress induced forms (Manicardi *et al.*, 1995; Sakkas *et al.*, 1999). The last one is the oxidative stress mechanism that has been studied extensively, and is caused by the overproduction of reactive oxygen species (ROS) (Sharma and Agarwal, 1996; Aitken and Krausz, 2001; Agarwal and Said, 2005; Lewis and Aitken, 2005). All mechanisms described above, either individually or together, have some bearing on the presence of abnormal spermatozoa in the ejaculate, and they may or may not be interrelated (Sakkas *et al.*, 2003).

Abnormal and dysregulated chromatin packing

Sperm chromatin structure, on contrary to somatic cells, is tightly compacted due to the unique associations between the DNA and sperm nuclear proteins. These nuclear proteins are predominantly comprised of protamines which are highly basic proteins. Thereafter the displacement of histones by transition proteins and then by protamines, the spermatid nucleus is remodelled and condensed in the final stages of spermatogenesis. The sperm DNA strands are tightly wrapped around the protamine molecules (about 50 kb of DNA per protamine) for forming tight and highly organized loops. The compaction and stabilization is organized by inter- and intramolecular disulphide cross-links between the cysteine-rich protamines.

More than two-thirds of the chromatin structure of human spermatozoa is thus packaged by protamines, only up to 15% of the DNA are less tightly compacted and packaged by histones. It has been shown that infertile men have an increased sperm histone:protamine ratio than fertile counterparts. This alteration of histone:protamine ratio, that is also called abnormal packing, increases susceptibility of sperm DNA to external stresses due to poorer chromatin compaction. Furthermore, complete deficiency of protamine has been demonstrated in about 5–15% of infertile men (Carrell and Liu, 2001). Recent studies also underlined the link between protamine deficiency and sperm DNA damage that resulted in poor fertilizing capacity (Nasr-Esfahani *et al.*, 2005). However there is a non-random selection of these histone-bound DNA sequences or genes in normal sperm DNA, and these are mainly presumed to be involved in fertilization and early embryo development (Tesarik *et al.*, 2002).

The mitochondrial DNA of human spermatozoa is a small, circular DNA which is not bound to special proteins. It has been demonstrated that sperm motility is directly related to the mitochondrial volume within the sperm mid-piece. The mitochondrial DNA exhibits a high rate of mutation or deletions that have been associated with reduced sperm motility. The inheritance of mitochondrial DNA is primarily maternal and only in 1% of cases paternal transmission of mitochondrial DNA mutations have been reported (Schwartz and Vissing, 2002).

The role of apoptosis of human spermatozoa in DNA damage

Apoptosis can be postulated to have two putative roles during normal spermatogenesis: limitation of the germ cell population to numbers that can be supported by the Sertoli cells and, possibly, selective depletion of abnormal spermatozoa (Sakkas *et al.*, 2003). Throughout life, apoptosis eliminates cells that are useless or potentially dangerous to the host such as aged, infected, injured or mutated cells. In normal conditions, more than half of the potential number of the mature germ cells is lost, mostly due through apoptosis of spermatogonia and spermatocytes (Dunkel *et al.*, 1997).

During apoptosis the cells shrink and exhibit several typical features, including cell membrane disruption, cytoskeletal rearrangement, nuclear condensation, and intranucleosomal DNA fragmentation (Kaufmann and Hengartner, 2001). Apoptosis in the human spermatozoa is a result of DNA strand breaks induced by a cascade of regulatory mechanisms with infertility (Høst *et al.*, 2000). The degradation of DNA into fragments approximately 185 bp and its multiples in size is one of the best characterized biochemical features of apoptotic cell death and is used as the basis for the commonly used labelling techniques for detecting apoptotic cells (Nagata, 2000). Apoptotic cells are usually rapidly taken up and degraded by neighbouring cells before their intracellular contents leak into the extracellular space. In contrast, acute accidental injury may lead to an uncontrolled form of cell death, called necrosis, which is characterized by swelling and bursting of the dying cells with an accompanying inflammatory response (Raff, 1998)

However, dysregulation of this physiological germ cell apoptosis thus itself might cause male infertility. Inappropriate