Reproductive BioMedicine Online (2015) 31, 100–107

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ARTICLE

DNA double strand breaks in human spermatozoa can be predictive for assisted reproductive outcome

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Abstract Sperm DNA status has been reported to predict fertility outcomes in infertile men. The terminal deoxynucleotidyl transferase-mediated dUDP nick-end labelling test (TUNEL) is the most widely used method to evaluate this; however, its prognostic value is still debated. One hundred infertile men undergoing intracytoplasmic sperm injection (ICSI) and 61 fertile men were tested for sperm parameters, sex hormones and sperm DNA status by chromatin tests (acridine orange, aniline blue, decondensation) and by direct assays (TUNEL and phosphorylated histone H2AX-γH2AX). In both groups, the prognostic value of each parameter to predict assisted clinical pregnancy was compared. Sperm parameters ($P < 0.05$ or $P < 0.01$), FSH levels ($P < 0.05$) and DNA status ($P < 0.05$ to $P < 0.001$) were significantly different in participants compared with controls. Among infertile men, 47 had positive and 53 had ICSI outcome. Both chromatin analysis and TUNEL test were unable to distinguish individuals who had successful outcomes from those who failed ICSI treatments. γH2AX percentage and γH2AX fragmentation index were significantly higher in sperm from non-pregnant compared with pregnant couples ($P < 0.05$ and $P < 0.01$). γH2AX assay is more predictive of ICSI outcome than TUNEL in infertile couples with male factor infertility.

KEYWORDS: double strand breaks, ICSI outcome, male infertility, sperm DNA fragmentation, γH2AX

http://dx.doi.org/10.1016/j.rbmo.2015.03.009

Introduction

The delivery of unaltered paternal genomes by sperm cells is one of the fundamental steps of transmitting a correctly functioning genome to the new generation. The formation of haploid spermatozoa through spermatogenesis and spermiogenesis enables fertilization to occur. This process, which is continuous and conserved among many species, is fundamental for male fertility. During spermiogenesis, the nucleosomal histone-based structure is nearly completely substituted by a protamine-based structure (Rathke et al., 2014). This replacement is likely to be facilitated by incorporation of histone variants, post-translational histone modifications and chromatin-remodeling complexes, as well as transient DNA strand breaks. The consequences of mutations have revealed that a protamine-based chromatin is essential for fertility. Today, male infertility is a problem affecting about one-half of all infertile couples and the causes are often due to errors that occur during spermiogenesis and other factors, including abortive apoptosis, altered histone protamine substitution, or both, during the remodelling of sperm chromatin occurring at spermiogenesis and DNA fragmentation. These conditions can be induced by reactive oxygen species potentially originated by many factors, including radiotherapy, chemotherapy, infections and lifestyles (Harrouk et al., 2000; Sakkas and Alvarez, 2010).

To analyse the DNA damage, routine semen analysis is insufficient, and more specific tests are required. Over the years, an increasing number of tests have been developed to assess sperm DNA status (Ribas-Maynou et al., 2013). Although direct methods, such as the single-cell gel electrophoresis assay (Comet Test) and the TdT (terminal deoxynucleotidyl transferase)-mediated dUTP nick-end labelling test (TUNEL), directly detect the strand breaks of DNA, other tests including, for example, sperm chromatin dispersion test, acridine orange test, sperm chromatin structure assay (SCSA), aniline test and condensation test, analyse the susceptibility of sperm DNA to breakage (Chohan et al., 2006).

Recently, it has been suggested that information on sperm DNA type damage should be mandatory to predict fertility outcome, considering that the oocyte seems to be more prone to repair single strand breaks than double strand breaks (DSB) (Sakkas and Alvarez, 2010). Some investigators, however, have argued that some kinds of DSB seem to be even easier to repair than single (Ribas-Maynou et al., 2012, 2014). Nonetheless, it is well accepted that the ability of the oocyte to repair sperm DNA damage depends on its cytoplasmic and genomic qualities, which are particularly related to the age of patients (Braude et al., 1988). Little is known about what happens when DSB are present in spermatozoa during the process of fertilization. It has been proposed that when the fertilizing spermatozoa has nuclear DSB,the zygote could fail to repair altered DNA, leading to genetic alterations like mutations, which could later block or alter embryo formation and development (Derijck et al., 2008). Therefore, interest is growing in identifying reliable methods for evaluating the presence of DSB in mature human spermatozoa.

Recently, a new test exploring the phosphorylation of the histone H2AX has been introduced for the detection of DSB in somatic cells (Fernandez-Capetillo et al., 2004; Hernández et al., 2013; Kinner et al., 2008). It is known that, in the presence of DSB, some protein-kinases, such as ataxia telangiectasia mutated protein, can induce H2AX phosphorylation. Phosphorylation of a serine residue in position 139 of the extreme C-terminus of H2AX, leads to the formation of a phosphorylated form of the histone H2AX (γH2AX) (Revet et al., 2011). In mature spermatozoa, the replacement of histones with protamines is not complete, and a small fraction of DNA (about 15%) remains bound to histones and also contains the histone variant H2AX (Gatewood et al., 1990; Oliva and Dixon, 1991; Wykes and Krawetz, 2003). A higher presence of γH2AX has been demonstrated in human mature spermatozoa after exposure to oxidative stresses induced by hydrogen peroxide or by incubation of ejaculated spermatozoa with mutagens such as Adriamycin (Li et al., 2006, 2008). In these studies, DNA fragmentation evaluated by COMET test, revealed a close relationship with the degree of histone H2AX phosphorylation examined by fluorescence microscopy. Moreover, γH2AX staining is a more sensitive indicator of DSB than COMET assay, when DNA damage is induced by a chemical reagent at low concentrations or for short incubation time (Li et al., 2006). Phosphospecific antibodies recognizing the phosphorylated S-139 residue of H2AX (γH2AX), are able to detect DSB and also to document local formation of distinct foci in their vicinity (Fernandez-Capetillo et al., 2004). Therefore, the assessment of H2AX phosphorylation is now considered a viable and valid test in the study of DSB in somatic cells (Gatewood et al., 1990; Revet et al., 2011), and has also recently been used in human spermatozoa (Li et al., 2006). The particular sperm DNA structure and conformation does not allow detection of H2AX phosphorylation through the observation of foci; therefore, a flow-cytometric analysis is needed to detect and quantify the presence of DSB spermatozoa. Li et al. (2006) have previously shown that spermatozoa, a terminally differentiated cell with no capacity to initiate new transcription-translation, is able to phosphorylate nuclear histone H2AX originating γH2AX. As previously reported in somatic cells, the same investigators showed that, in sperm exposed to oxidative stress through incubation with H_2O_2, the phosphorylation of H2AX is strictly dependent on phosphatidylinositide 3-kinases (PIK3), a family of enzymes involved in cellular functions such as cell growth, proliferation, differentiation, motility, survival and intracellular trafficking. In fact, using wortmannin, an inhibithor of PIK3 family, H2AX phosphorylation did not occur (Gatewood et al., 1990; Li et al., 2006).

Presently, TUNEL test is the most widely used method to evaluate sperm DNA integrity before assisted reproduction techniques worldwide. This method is unable to distinguish both the presence of single or DSB and to quantify these alterations. So far, no studies have evaluated the clinical relevance of DSB analysed by γH2AX test on human spermatozoa and, in particular, their role in assisted reproductive outcome. In the present study, the sperm DNA status was analysed by chromatin tests in semen samples from proven fertile and infertile patients. Moreover, in the same samples the DNA fragmentation was evaluated by TUNEL and γH2AX test comparing patients who had positive or negative fertility outcome within three cycles of intracytoplasmic sperm injection (ICSI).